AMINOGLYCOSIDE RELATED NEPHROTOXICITY IN CYSTIC FIBROSIS

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Acknowledgments

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DECLARATION

This thesis is the result of my own work. The work was carried out at the Liverpool Adult Cystic Fibrosis Unit and the attached Research Laboratory, Liverpool Heart and Chest Hospital NHS Trust and at the Microbiology Department, Royal Liverpool & Broadgreen University Hospitals NHS Trust. All the work was performed by the author with the exception of sputum *P. aeruginosa* colony counts which were done by the staff at the Diagnostic Laboratory, Microbiology Department, Royal Liverpool University Hospital.

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LIST OF ABBREVIATIONS

AAP	alanine aminopeptidase
AE	adverse events
AKI	acute kidney injury
aMDRD	abbreviated Modified Disease and Renal Diet formula
ARF	acute renal failure
BMI	body mass index
β2M	β2-microglobulin
BP	blood pressure
BSA	body surface area
°C	degrees Celsius
CGF	Cockcroft and Gault formula
CCl	creatinine clearance
CF	cystic fibrosis
CFTR	cystic fibrosis transmembrane conductance regulator
cfu	colony forming unit
CI	confidence interval
CKD	chronic kidney disease
COL	colistin (colistin sulphomethate sodium)/colomycin®
Cr	creatinine

CRP	C-reactive protein
DTPA	diethylenetriamine penta-acetic acid
eCCl	estimated creatinine clearance
EDTA	ethylene diamine tetra acetate
ESRD	end stage renal disease
FEV1	forced expired volume in 1 second
FOM	fosfomycin (fosfonomycin)
FVC	forced vital capcity
GFR	glomerular filtration rate
hr	hour
IV	intravenous
Kg	kilogram
kg/m^2	kilogram/metre squared
K/DOQI	Kidney Disease Outcomes and Quality Initiative
LES	Liverpool epidemic strain
mCCl	measured creatinine clearance
MDRD	Modified Disease and Renal Diet formula
µg	microgram
Mg^{2+}	magnesium ion
MIC	minimum inhibitory concentration

µmol	micromole
mmol	millimole
MSSU	mid stream sapmle of urine
MRSA	methicillin resistant *Staphylococcus aureus*
NAG	N-acetyl-β-D glucosaminidase
NSAIDS	non-steroidal anti-inflammatory drugs
P. aeruginosa	*Pseudomonas aeruginosa*
PKD	polycystic kidney disease
PO	per os
/l	per litre
SD	standard deviation
SCr	serum creatinine
TIN	tubulointerstitial nephritis
TNS	tobramycin nebuliser solution (TOBI®)
TOB	tobramycin
U&E	blood urea, serum creatinine and electrolytes
UKCKDG	UK chronic kidney disease guidelines
USS	ultrasound scan
vs	versus
WCC	white cell count

Abstract

Aminoglycoside related nephrotoxicicty in cystic fibrosis

Mohamed Al-Aloul

The last few years witnessed increasing interest in renal function of CF patients. The CFTR is expressed in all parts of the kidney, but traditional views were that, apart from nephrolithiasis, no specific pathology resulted and deranged renal function was rare.

The *P. aeruginosa* Liverpool Epidemic Strain (LES) is endemic in our clinic and often only predictably sensitive to aminoglycosides (tobramycin) and polymyxins (colistin), both potentially nephrotoxic agents. Several reports of drug-related acute renal failure in CF were cited. I present our own case series, the first to be published in the adult CF literature: all were related to IV aminoglycoside therapy. Subsequently, in a cross sectional survey of 80 consecutive stable patients attending the outpatient clinic, I identified a high level (42%) of previously unrecognised background renal impairment using CCl as an estimate of GFR from 24-hour timed urine collections. This was significantly associated with lifetime exposure to IV aminoglicosides. IV colistin, at recommended doses, did not appear to be independently nephrotoxic but potentiated the cumulative renal injury of aminoglycosides in combination. Due to practical shortfalls with urine collections, use of formulae to estimate GFR is advocated. Several are employed in chapter 6; they correlate to varying degrees with CCl measurement but uniformly tended to overestimate GFR in the subgroup with impaired renal function, limiting their practical utility as a screening tool for renal dysfunction in this patient group.

The primary toxic effect of aminoglycosides and polymixins is on the proximal tubular epithelium. Release of tubular enzymes and proteins in urine is a sensitive method of quantifying acute subclinical structural and functional changes due to drug-induced tubular injury and monitoring its recovery. Using these assays I was able to confirm that frequent aminglycoside exposure, without sufficient time for complete tubular recovery, is one plausible mechanism underlying the cumulative toxicity documented earlier. In contrast patients whose tubules recover fully before subsequent drug challenge are likely to handle re-exposure with less incremental injury. "Aminoglycoside-holidays" are proposed but the limited antibiotic choice available to treat LES creates a need for other renal sparing startegies. TOBI, a nebulised formulation of tobramycin delivers the drug directly to the site of infection whilst minimising systemic bioavailabilty. FOM, a unique antibiotic with antipseudomonal properties, is shown in animal models to protect against aminoglycoside induced disruption of the proximal tubular lysosomal system. Compared with IV TOB and COL combination in randomised cross over trials, I was able to demonstrate similar clinical responses in the treatment of acute pulmonary exacerbations with a combination of either TOBI and IV COL or IV TOB, COL and FOM with the added benefit of attenuated acute renal injury, reflected in lower levels of enzymuria and proteinuria. The potential for long term nephroprotection with these approaches needs further examination with longitudinal multicentre trials employing sensitive and specific renal tubular assays.

CHAPTER ONE

Introduction

1.1 CYSTIC FIBROSIS (CF)

Cystic fibrosis (CF) is an autosomal recessive disorder and the most common inherited lethal disease of Caucasian populations, with an incidence of about 1 in 2500 live births and a carrier frequency of 1 in 25 (Bye et al, 1994). The disease affects approximately 30,000 individuals in the U.S. and 60,000 individuals worldwide (Gibson et al, 2003a). The clinical picture is dominated by disease in the respiratory, gastrointestinal and reproductive tracts. The main characteristics of CF are recurrent bacterial infections in the lower respiratory tract, malabsorption due to exocrine pancreatic insufficiency, and male infertility due to absence or stenosis of the vas deferens (Koch and Hoiby, 1993). Pulmonary disease is responsible for most of the morbidity in CF and is the cause of death in over 90% of patients (Davies, 2002). The quality of life and life expectancy of patients with CF have been greatly enhanced over past decades due to better notions of symptomatic treatment strategies and as a result, patients with the disease now often live beyond the third decade. However, life with CF is still shadowed by the prospect of premature death due to respiratory failure resulting from recurrent bacterial infections.

1.2 THE MOLECULAR BASIS OF CF

Cystic fibrosis is caused by mutations in the CF gene which has been identified, cloned and sequenced, and its protein product has been analysed (Kerem et al, 1989; Riordan et al, 1989; Rommens et al, 1989; Zielenski et al, 1991). The gene is 250 kb long, identified in the q21-31 region of the long arm of chromosome 7, and encodes a multifunctional transmembrane protein of 165-kD containing 1480 amino acids termed the cystic fibrosis transmembrane conductance regulator (CFTR) and expressed in a variety of secretory as well absorptive epithelia including renal tubules (Rommens et al, 1989; Berger et al, 1991; Riordan, 1993).

In the apical plasma membrane, CFTR is part of a multiprotein assembly consisting of two transmembrane (anchoring) domains (TMDs) and two nucleotide-binding domains (NBDs), separated by a larger regulator domain (RD) containing multiple phosphorylation sites (figure 1.6.1) (Riordan, 1993; Foskett, 1998). It is now established that CFTR functions as a 3'5'-cyclic monophosphate (cAMP)-activated Cl^- channel that modulates a series of intracellular functions by a complex process involving both phosphorylation by 3'5'cAMP-dependent protein kinase A (PKA) and the interactions of ATP with the nucleotide binding domain (Anderson and Welsh, 1992; Morales et al, 1999). The CFTR also has a voltage dependent low conductance for Cl^- (9pS)(Berger et al, 1991).

The carboxyl terminal (TRL) of CFTR is anchored in the cytoskeleton, in close proximity to a number of important proteins such as signal transduction proteins and other ion channels (Riordan, 1993; Foskett, 1998; Rowe et al, 2005). On one hand, these associated proteins influence CFTR functions and are potential modifiers of the CF phenotype which varies substantially among patients with the same mutations in CFTR. Reciprocally, and through interaction with these neighbouring protein complexes, CFTR plays an important role in intracellular vesicular acidification (Barasch et al, 1991), exocytosis, protein processing and traffic (Morris and Frizzell, 1994), secretion of ATP (Winter et al, 1994), down regulation of transepithelial sodium transport through the epithelial Na channel (ENaC), regulation of calcium-activated chloride channels and potassium channels [the outwardly rectifying Cl^- channel (ORCC) and the renal secretory K channel (ROMK-2)] (Schwiebert et al, 1995; Stutts et al, 1995; Ismailov et al, 1996; McNicholas et al, 1996).

Over 1000 mutations in this gene have been described so far. These mutations can be classified on the basis of the mechanism by which they are believed to cause disease into six classes: absence of synthesis (class I), defective protein maturation

and premature degradation (class II), disordered regulation (class III), defective chloride conductance (class IV), reduced number of CFTR transcripts due to a promoter or splicing abnormality (class V), and defective CFTR stability at the cell surface (class VI) (Rowe et al, 2005). The most common mutation worldwide, termed ΔF508, is categorised as a class II defect. CFTR with the ΔF508 mutation lacks a phenylalanine residue at position 508. The defective protein is rapidly recognised as misfolded and is degraded shortly after synthesis before it can reach its site of action at the cell surface. It is present in approximately 70% of defective CFTR alleles and in 90% of patients with CF in the United States (Rowe et al, 2005), but its frequency varies between ethnic groups from 30% in Ashkenazi Israeli patients to 88% of Danish (Koch and Hoiby, 1993).

With the description of so many varied mutations in CFTR, attention was drawn to the relation between genotype and the phenotypic manifestations of the disease. Generally, class I-III mutations, which are the most common, are associated with pancreatic insufficiency, whereas patients with rarer class IV-VI mutations typically do not have insufficiency (Ratjen and Doring, 2003). Although several mutations are known to be associated with less severe pancreatic disease, the association of genotype with the severity of lung disease is less clear. No relation between genotype and the course of lung disease was found in a study that included eight CFTR mutations occurring in 72% of patients with CF from 14 countries. The A455E mutation (class IV), a common mutation causing CF in the Netherlands, has been found to correlate with mild pulmonary disease, probably due to residual CFTR ion-channel activity (Gan et al, 1995). In Denmark, more aggressive pulmonary disease was found in patients who were homozygous for ΔF508 than in patients who were compound – i.e., who had ΔF508 on one chromosome and another rarer mutation on the other (Johansen et al, 1991). In a retrospective cohort study that included approximately 18,000 patients using the US Cystic Fibrosis Foundation National Registry, the mortality rates for the 11 most common genotypes heterozygous for ΔF508 was compared with those homozygous for ΔF508. Distinct genetic subgroups

were found to be associated with mild clinical manifestations and low mortality (McKone et al, 2003). However, substantial differences in clinical severity are often observed in patients with the same CFTR mutations. For example, the course of pulmonary disease often differs strikingly among CF siblings, and the progression of disease is highly dependent on treatment, which can vary from one CF centre to another (Koch and Hoiby, 1993). The wide phenotypic variation in patients homozygous for ΔF508, and differences in chloride conductance between monozygous and dizygous twins, suggest that environmental factors and/or genes other than CFTR, modify progression and severity of the disease (Ratjen and Doring, 2003).

There is widespread agreement that defective ion transport, salt homeostasis, or both, due to the absence of normal functioning CFTR are intimately linked to organ damage in CF. The precise molecular mechanism, however, is unknown. Different models have been proposed to explain the salt and fluid metabolism abnormalities observed in the CF lung (Gibson et al, 2003a; Rowe et al, 2005). In the "low-volume model", the absence of CFTR leads to overactivity of sodium absorption through epithelial sodium channels (ENaC) and chloride absorption continues through non-CFTR pathways. The resulting sodium and water hyperabsorption causes the dehydration of airway surfaces, defective mucociliary transport and accumulation of thick mucus leading to airway obstruction. Similar mechanisms explain the increase in sweat Cl^- concentration and thick secretions in the bowel lumen and the pancreatic ducts result in intestinal obstruction and pancreatic insufficiency (Davis et al, 1996). In the "high-salt model", the absence of CFTR restricts reabsorption of chloride, the transepithelial potential difference becomes hyperpolarised and there is elevated amount of salt in the airway-surface liquid. It has been suggested that the raised luminal salt concentration would inactivate endogenous antimicrobial peptides and thereby predispose patients to bacterial infections (Smith et al, 1996). Regardless of which model better explains the

pulmonary complications in CF, there is a strong consensus that the airways lack the normal ability to secrete chloride through CFTR (Rowe et al, 2005).

1.3 CFTR IN THE KIDNEY

In mammals, the kidneys are responsible for the maintenance of extracellular sodium chloride (NaCl) concentration which in turn regulates the extracellular fluid volume (ECFV) and blood pressure. Na^+ and Cl^- are reabsorbed along the nephron, reaching over 99% of the filtered load under low salt diets. Cl^-, the predominant anion in the glomerular ultrafiltrate, is reabsorbed along the nephron either by trans- or para-cellular pathways (Berry and Rector, 1991). Trans-cellular transport of Cl^- involves several membrane proteins and channels including the CFTR.

In spite of the injury in several organs, patients with CF do not develop major renal dysfunction although they have reduced renal excretion of NaCl and decreased capacity to dilute and concentrate urine (Stenvinkel et al, 1991; Donckerwolcke et al, 1992). These patients also have an enhanced excretion of penicillins and aminoglycosides in urine (Strandvik et al, 1989; Bates et al, 1997). The impaired salt reabsorption by the kidney could be related to changes in ECFV caused by excessive losses of NaCl in sweat and faeces. However, decreased NaCl renal excretion might also result from a primary defect in renal tubular function caused by mutations in CFTR.

Several studies have demonstrated the presence of CFTR in the kidney, and its mRNA has been detected in all nephron segments by reverse transcription PCR (Riordan et al, 1989; Morales et al, 1996). CFTR was detected in proximal tubule, thin limb of Henle's loop, the lumenal membrane of distal tubule, cortical collecting duct, and the inner medullary collecting duct by immunocytochemistry (Crawford et al, 1991). Patch clamp analysis has also confirmed its presence in proximal and

distal tubules and in cortical and inner medullary collecting ducts (Husted et al, 1995; Letz and Korbmacher, 1997). CFTR is highly expressed during the early stages of kidney development. At gestational age 12 weeks kidney CFTR is confined to the apical membrane of the ureteric bud-derived collecting tubules, and its presence in proximal tubule cytoplasm is first seen at 16 weeks gestation (Devuyst et al, 1996).

1.3.1 CFTR in proximal tubule

Although immunolocalisation studies indicate an apical expression of CFTR in the proximal tubules (Crawford et al, 1991), patch clamp studies localise its activity at the basolateral membrane (Rubera et al, 1998; Simpson et al, 2005). The function of CFTR in the proximal tubules is uncertain because the reabsorption of NaCl in this segment of the nephron is increased in CF, rather than decreased as would be expected if CFTR mediates Cl^- absorption (Stenvinkel et al, 1991; Bates et al, 1997). The increase in CF kidney Cl^- reabsorption could be associated with the reduced ECFV or with a reduced NaCl reabsorption in the thick ascending limb of Henle and distal tubule, both found in CF (Strandvik et al, 1989; Donckerwolcke et al, 1992).

On the other hand, in CF the defective CFTR channel causes an increase in penicillin and aminoglycosides excretion in the proximal tubule. One hypothesis for this phenotype is that the abnormal CFTR decreases Cl^- reabsorption, which increases Cl^- in the tubular lumen. This Cl^- will move into the cell in exchange for those drugs, which increases their clearance (Woodland et al, 1998).

1.3.2 CFTR in Henle's loop and distal nephron segments

The mRNA for CFTR was found in the thin ascending limb of Henle's loop, but no protein was identified in this nephron segment by immunolocalisation or patch clamp studies (Crawford et al, 1991; Devuyst et al, 1996). The CFTR protein has not

been detected in the thick ascending limb of Henle's loop by immunocytochemistry, although in patch clamp studies a Cl^- channel with a low conductance (9pS), dependent on ATP and Mg^{2+}, was found in the basolateral membrane (Berger et al, 1991). This channel was nominated pseudo CFTR, since it is still functional in CFTR knockout mice (Marvao et al, 1998).

Distal convoluted tubule CFTR was detected by immunolocalisation and patch clamp studies in the apical membrane (Rubera et al, 1998; Barriere et al, 2003). Probably Cl^- is secreted into the luminal fluid through CFTR in response to the electrochemical gradient generated by the Na^+/Cl^- co-transporter. CFTR in this segment may also facilitate HCO_3^- secretion by mediating the recycling of Cl^- through the Cl^-/HCO_3^- exchanger in the apical membrane (Tauc et al, 1996; Rubera et al, 1999). CFTR protein was not found in the collecting tubule, in the α and β intercalated cells of collecting ducts or in the outer medullary collecting duct (OMCD) (Crawford et al, 1991; Todd-Turla et al, 1996).

1.3.3 CFTR in collecting ducts

In cortical collecting ducts (CCD) the CFTR protein was only identified in the apical membrane of the principal cells (Huber et al, 1998; Bens et al, 2001). There is experimental evidence that Cl^- absorption in this nephron segment is through the paracellular pathway following the electrochemical gradient (Koeppen and Stanton, 1992). However, depending on the electrochemical driving force for Cl^- movement across the apical membrane CFTR channel, this ion could either be absorbed or secreted (Ling et al, 1994). This Cl^- movement is dependent on Na^+ absorption through the ENaC channel and may be controlled by hormones like aldosterone and arginine-vasopressin (Duong Van Huyen et al, 1998).

In the inner medullary collecting duct (IMCD) CFTR plays an intriguing role. The CFTR channel is expressed at the apical membrane in this nephron segment, but an alternative splice form of this molecule, the TNR-CFTR was also found (Morales et al, 1996). Electrophysiological studies support the view that Cl^- secretion through the CFTR follows the electrochemical driving force across the apical membrane (Husted et al, 1995; Kizer et al, 1995). The Cl^- secretion occurs by a two step process, first involving the uptake of Cl^- across the basolateral membrane by Cl^-/HCO_3^- exchange and $Na^+/K^+/2Cl^-$ co-transport, then the efflux of Cl^- across the apical membrane through CFTR channel (Rocha and Kudo, 1990; Kizer et al, 1995; Moyer et al, 1995; Letz and Korbmacher, 1997). Arginine vasopressin (AVP) stimulates Cl^- secretion in IMCD by increasing 3'5'-cAMP which activates PKA which in turn activates CFTR channel (Moyer et al, 1995). The role of AVP on Cl^- secretion may be related to the fact that Na^+ secretion follows that of Cl^-, inducing natriuresis and chloruresis to maintain a normal plasma osmolality during dehydration, as observed *in vivo* in rats (Luke, 1973).

1.3.4 TNR-CFTR, the renal splice variant of CFTR

TNR-CFTR, a splice variant isoform of CFTR gene is associated with specific small endosomal populations highly expressed in the renal medulla (Morales et al, 1996). Functional studies with TNR-CFTR, expressed in *Xenopus oocytes* or mammalian cells, showed cAMP-dependant single Cl^- channel properties like those of the wild type CFTR, but with lower efficiency (Morales et al, 1996). In medullary collecting ducts the TNR-CFTR protein and mRNA expression is present during embryonic life, increasing during fetal kidney development and reaching the highest level at birth (Devuyst et al, 1996). The specific function of the TNR-CFTR is not clear and does not seem to be related to Cl^- secretion. Because it is found in abundance in intracellular vesicles in the cytoplasmic compartment, it may be involved in vesicular trafficking.

1.3.5 CFTR and polycystic kidney disease (PKD)

CFTR expression and chloride channel function is normal in PKD, an autosomal dominant genetic disease (Hanaoka et al, 1996; Sullivan et al, 1998). This renal disorder is characterised by the presence of multiple epithelial cysts with epithelial cell proliferation and apical fluid secretion. The cyst enlargement in PKD kidney is thought to involve inappropriate polarized secretion of Na^+ ions into the tubule lumen due to the mispolarisation of the (Na^+ + K^+)-ATPase pump in the cyst apical membrane (Wilson, 1997). Na^+ and Cl^- contents in cyst fluid are abnormally high, and addition of cAMP to PKD cyst epithelia in culture increases fluid secretion and ATP release into the cyst fluid. These findings could be due to the CFTR, since it is well known that this channel transports both Cl^- and ATP (Sullivan et al, 1998) and suggest involvement of CFTR in PKD cyst fluid secretion and enlargement.

1.3.6 CFTR and other conductances

Cl^- transport by CFTR in renal epithelia represents a high-energy expenditure, since this channel requires ATP hydrolysis (Winter et al, 1994) and its conductance is too low (9pS) to produce fast absorptive or secretive fluxes. One possible role for CFTR in the kidney is the activation or inhibition of other channels like ORCC, ENaC and ROMK2 (Schwiebert et al, 1995; Ismailov et al, 1996; Morris, 1999; Wang, 1999). It has also been shown that AVP produces an increase of Cl^- secretion due to transcriptional enhancement in the expression of CFTR and other transporters in rat cortical collecting duct cells (Djelidi et al, 1999).

Recent studies form Morales' group showed that in homozygous Brattleboro rats, a strain of Long-Evans rats carrying an autosomal recessive mutation that results in a deficiency of AVP secretion in the plasma, the expression of CFTR mRNA was low in the renal cortex and medulla but returned to normal after AVP reposition. The mRNA of CFTR was increased in the medulla of dehydrated Wistar rats and no

variation was observed in the cortex. The modulation of CFTR by the main hormone involved in the regulation of fluid osmolality suggests that this Cl^- channel plays a role in ECFV regulation (Morales et al, 2001).

In conclusion, the studies discussed above show the importance of the CFTR channel in the kidney. Besides its function in Cl^- transport, CFTR modulates different epithelial conductances, such as channels for sodium (ENaC), potassium (ROMK2) and chloride (ORCC), probably by mediating ATP transport. Although very well studied in other organs, the function of CFTR is not fully understood in the kidney and further studies are crucial to understand its role in renal physiology.

1.4 CYSTIC FIBROSIS AND RENAL DISEASE

As mentioned above, although the CFTR gene is expressed in all nephron segments (Morales et al, 2000), clinically significant primary renal disease, with perhaps the exception of nephrocalcinosis, is not a recognised feature of CF. However, patients with CF are at risk of developing secondary renal disease either in association with chronic bronchial sepsis or as a complication of other organ involvement, for example pancreatic disease resulting in diabetes or, more commonly, as a result of nephrotoxic medications. As the prognosis of patients with CF improves, renal complications may become more prevalent and assume greater importance.

1.4.1 Nephrocalcinosis and nephrolithiasis

CF is associated with an increased risk of nephrocalcinosis and nephrolithiasis. In an autopsy study of 38 patients with CF, microscopic nephrocalcinosis was present in 35, including one still-born infant and two neonates (Katz et al, 1988). The incidence of nephrolithiasis in CF ranges from 3% to 6.3% (Strandvik and Hjelte, 1993; Chidekel and Dolan, 1996; Matthews et al, 1996; Hoppe et al, 1998; Perez-Brayfield

et al, 2002; Gibney and Goldfarb, 2003) compared to 1-2% in age-matched controls without CF (Gibney and Goldfarb, 2003). The majority of renal stones are composed of calcium oxalate. In a study of 13 CF patients with renal stones, patients presented at a mean age of 27 years, with flank pain as the presenting symptom in 69% of cases (Perez-Brayfield et al, 2002).

It is tempting to speculate that the genetic defect in the CFTR protein causes nephrocalcinosis/nephrolithiasis, particularly since Dent's disease, an X-linked proximal renal tubular disorder characterised by hypercalciuria, nephrocalcinosis, nephrolithiasis and low molecular weight proteinuria, has now been ascribed to a renal specific Cl^- channel defect (Lloyd et al, 1996). However, hypercalciuria is not a universal finding in patients with CF, which implies that stone formation is caused by factors other than the underlying genetic defect (Matthews et al, 1996; Gutknecht, 2001). Such factors include low urine volume, hypercalciuria (which is exacerbated by furosemide therapy, treatment with steroids and prolonged periods of immobilisation), hypocitraturia (von der Heiden et al, 2003), hyperoxaluria and hyperuricosuria (Turner et al, 2000; Perez-Brayfield et al, 2002; Terribile et al, 2006). The data regarding calcium and urate secretion are conflicting. Some authors report that hypocalciuria is protective against nephrolithiasis in CF patients (Bohles and Michalk, 1982) and suggest that calcium supplements, given for example for CF-associated low bone mineral density, may increase the risk of stone formation. A greater degree of consensus exists for hyperoxaluria and hypocitraturia as risk factors for renal stones. Hyperoxaluria is often alimentary in origin and is associated with pancreatic insufficiency and fatty acid malabsorption (Hoppe et al, 2005). It is aggravated by the reduction or disappearance of oxalate degrading bacteria in the gut, especially the bacterium *Oxalobacter formigenes* (Sidhu et al, 1998). In the latter study 71% of healthy volunteers were colonised with *O. formigenes* compared to only 16% of patients with CF. The intensive use of antibiotics in CF care may induce permanent gut decolonisation with *O. formigenes*, increased oxalate absorption and subsequent hyperoxaluria (Hoppe et al, 1998; Hoppe et al, 2005).

CF patients with urolithiasis warrant detailed metabolic evaluation, as correction of risk factors may decrease stone recurrence. Pancreatic enzyme dosage should be reviewed by a specialist dietician. Fluid intake should be increased and supplemental citrate prescribed for those with a proven history of urolithiasis. Stones may be passed in the urine with medical intervention or require extra corporeal shock wave lithotripsy or ureteroscopy with stone extraction (Perez-Brayfield et al, 2002).

1.4.2 IgA nephropathy

IgA nephropathy, although rare, is the most frequently reported glomerulonephritis in patients with CF (Melzi et al, 1991). In 1999, Stirati reported that four out of five renal biopsies performed for haematuria, proteinuria or reduced renal function showed IgA nephropathy (the fifth demonstrated amyloidosis) (Stirati et al, 1999). The co-existence of CF and IgA nephropathy may be coincidental, since both are relatively common disorders; however, there may be a real association because patients with CF have high circulating levels of IgA due to recurrent bacterial infections which, if deposited in the kidney, may cause a further immune response resulting in glomerulonephritis.

1.4.3 Vasculitis

Vasculitis, usually antineutrophil cytoplasmic antibody (ANCA) positive, has also been described in older patients with CF, some of whom have had clinically evident nephritis (Finnegan et al, 1989). ANCA has also been found in children with CF and recurrent infections but without clinical vasculitis or glomerulo-nephritis.

1.4.4 Amyloidosis

With improved patient survival secondary amyloidosis, a sequel of chronic infection, is being increasingly recognised as a serious complication of CF. In 1986 McGlennen et al (McGlennen et al, 1986) reviewed the literature and reported their autopsy findings from 1957 to 1983: there were only 16 cases reported in the literature but in their cohort of 33 patients, all older than 15 years at the time of death, 11 had evidence of systemic amyloidosis including renal involvement. Only one patient had presented clinically and the diagnosis was made on renal biopsy prior to death (which was due to renal failure) (McGlennen et al, 1986). Since then there have been several reports of patients with CF presenting with nephrotic syndrome due to amyloidosis (Gaffney et al, 1993; Waz et al, 1995). If this occurs, the prognosis is poor with nearly all dying within a year of clinical presentation, although there have been encouraging results in two patients treated with colchicine (Kuwertz-Broking et al, 1995).

1.4.5 Diabetic nephropathy

More than 30% of CF patients have insulin-dependent CF related diabetes (CFRD) by early adult life (Brennan et al, 2004). Although there is probably a lower prevalence of renal complications in patients with CFRD compared with non-CF patients with other forms of diabetes (Schwarzenberg et al, 2007), it is estimated that 30-50% of those with CFRDM will progress to diabetic nephropathy within 5-10 years of diagnosis, with a proportion ultimately requiring renal replacement therapy (Sullivan and Denning, 1989; Magryta et al, 1999). It is therefore inevitable that with prolonged survival more patients with CF will develop diabetic nephropathy with the characteristic histological changes of nodular glomerulosclerosis.

The combination of long-standing diabetes mellitus, poor glycaemic control, plus pathophysiological features specific to CF (though not yet elucidated) may

contribute to the development of microangiopathy (Sullivan and Denning, 1989). Indeed, Westall et al (Westall et al, 2004) described 3 adult CF patients with renal dysfunction, who on renal biopsy had histological changes characteristic of diabetic nephropathy (Kimmelstiel-Wilson nodules) in the absence of abnormal glucose metabolism (using standard biochemical diagnostic criteria: all patients had normal fasting plasma glucose levels, oral glucose tolerance tests and measurements of HbA1c). They speculated that the pro-inflammatory cytokine profile, typical of CF, predisposes to the lesions described. Furthermore, Van den Berg et al (van den Berg et al, 2008) reported, in a case control study, that despite a similar overall burden of microangiopathic complications in patients with CFRD and non-CF type 1 insulin-dependent diabetics, there was a higher prevalence of microalbuminuria in the CFRD group even after correcting for blood pressure and level of glycaemic control (21% vs. 4%, P=0.003). This may reflect the influence of other CF-related factors on renal function.

Diabetic microangiopathy causes significant morbidity in patients with multisystem involvement, including blindness, glaucoma, hypertension, and renal failure (Sullivan and Denning, 1989; Magryta et al, 1999). In a non-transplanted cohort of CF diabetics (median duration of CFRD of 20 years) nine out of 29 patients had hypertension, three had microalbuminuria, and one had elevated creatinine. None had macroalbuminuria (Andersen et al, 2006). The presence of fasting hyperglycaemia increases the risk of microalbuminuria (Schwarzenberg et al, 2007) and the whole spectrum of renal abnormalities attributable to CFRD is more common in CF diabetics who also receive lung transplantation (Andersen et al, 2006). In another case control study, the microvascular complications shown by patients with CFRD were similar to those seen in patients with type 1 diabetes but with a lower prevalence of retinopathy and a higher prevalence of microalbuminuria. The latter may reflect the influence of other cystic fibrosis-related factors on renal function (van den Berg et al, 2008).

These observations suggest that routine screening for diabetes and its microangiopathic complications in the population with CF, as well as optimal control of hyperglycaemia, is warranted (Brennan et al, 2004). Although not yet adopted in all CF clinics, diabetologists are increasingly engaged in the multidisciplinary approach to management of people with CF (Andersen et al, 2006; van den Berg et al, 2008).

1.4.6 Drug-related nephrotoxicity

Nephrotoxic drugs are the commonest cause of renal injury in patients with CF. Potential nephrotoxicity may be exacerbated by combination drug therapy and by salt depletion (Bennett, 1997).

1.4.6.1 Aminoglycosides

Aminoglycoside antibiotics have an important role in the treatment of *Pseudomonas aeruginosa* infections. In common with other renally excreted drugs, serum concentrations of aminoglycosides are reduced in patients with CF, who consequently require high doses to achieve therapeutic drug levels and ensure clinical efficacy. Woodland (Woodland et al, 1998) has proposed that disordered CFTR expression at the apical surface of proximal tubular epithelial cells results in decreased Cl^- reabsorption from the proximal renal tubular fluid. This is compensated by increased exchange of Cl^- for organic anions thereby increasing tubular secretion of these organic anions including anionic drugs (Woodland et al, 1998). Aminoglycoside nephrotoxicity typically presents with non-oliguric acute renal failure and minimal abnormalities on urinalysis. It is thought to be caused by the accumulation of drug within the lysosomes of proximal renal tubular cells and results in acute tubular necrosis (ATN). It is reversible once aminoglycoside therapy has been discontinued, although tubular damage may continue for several days after cessation because of continued high parenchymal levels. Aminoglycoside nephrotoxicity is related to the dose and duration of therapy, as well as the level of renal function *before* and *during* treatment. CF patients are therefore at particular risk for aminoglycoside toxicity since they require high doses of antibiotics to

achieve therapeutic drug levels and often for prolonged courses. It is therefore essential that trough and peak serum drug levels are monitored carefully to both ensure therapeutic adequacy and avoid nephron- and oto-toxicity. Levels should be monitored until the correct drug dosage has been established. Drug levels should be repeated and renal function checked if there is any deterioration in the patient's condition.

1.4.6.2 Non-steroidal anti-inflammatory drugs

NSAIDS are used in CF for the management of CF-related arthropathy and chronic chest/back pain related to abnormal posture, chronic cough and chronic pleurisy. NSAID-induced renal dysfunction is rare in otherwise well individuals, but patients with CF are at increased risk because of chronic salt depletion and particularly if acutely dehydrated. This is because NSAIDS inhibit the enzyme cyclo-oxygenase thus blocking the production of vasodilator prostaglandins, causing renal vasoconstriction and acute renal dysfunction. It is therefore essential to monitor renal function in patients with CF taking NSAIDS, particularly if they are unwell (Orme, 1986; Lang et al, 1991; Lafrance and Miller, 2009).

1.4.6.3 Drug-induced nephrocalcinosis

As noted above, nephrocalcinosis and nephrolithiasis may be drug related. Loop diuretics (which may be used to treat right-sided heart failure in CF) and corticosteroids (which may be used in the management of chronic inflammatory lung disease) both increase calcium excretion and may cause or exacerbate nephrocalcinosis and nephrolithiasis (Randall et al, 1965; Barilla et al, 1978).

1.4.7 Tubulointerstitial nephritis (TIN)

TIN may complicate CF as an allergic reaction to drugs (including a large number of antibiotics) or to infection (for example, streptococcal infections). There are sometimes accompanying signs of systemic allergy such as rash or pain at a drug injection site. Non-specific symptoms such as malaise, fever, nausea and vomiting may be present and are occasionally misattributed to the underlying infection. These changes are also important since they may lead to dehydration, which increases the chances of nephrotoxicity due to aminoglycosides.

Laboratory findings in TIN include elevation of plasma urea and creatinine that may, in the early stages, be accompanied by hypokalaemia and hypophosphataemia (due to tubular dysfunction) and sometimes, a peripheral blood and / or urinary eosinophilia (Baker, 2002; Patzer, 2008; John and Herzenberg, 2009). Urinalysis will often reveal haematuria and proteinuria in addition to glycosuria. Ultrasonography usually reveals large, bright kidneys and renal biopsy, which is necessary to confirm the diagnosis, reveals a predominantly T cell interstitial infiltrate sometimes with tubular dilatation and areas of tubulointerstitial fibrosis(Sierra et al, 2007).

The treatment of TIN is primarily supportive, with dialysis therapy as indicated. Any suspected causative medication, which in a CF patient is most likely to be an antibiotic and most usually a β-lactam (Alexopoulos, 1998) antibiotic or a NSAID, should be discontinued. The use of corticosteroids in the management of acute TIN remains controversial but is probably justified, particularly in dialysis-dependent patients since, anecdotally, its use has been associated with rapid recovery of renal function. In infection-related acute TIN, specific treatment of the underlying infection is also indicated. It should be noted that in patients receiving combination drug therapy, such as a B-lactam antibiotic or a NSAID and an aminoglycoside, TIN-induced renal impairment will result in increased aminoglycoside levels and exacerbate aminoglycoside-related nephrotoxicity.

1.4.8 Lung transplantation

Lung transplantation predisposes patients with CF to renal disease (Schindler et al, 2001). There is an accelerated loss in the glomerular filtration rate compared to lung transplants for other pulmonary diseases (Broekroelofs et al, 2000; Hmiel et al, 2005). This post operative decline in lung function suggests that the patients' renal reserve may be impaired pre surgery. The patients are at risk of a further decline in renal function from the nephrotoxic effects of the postoperative immunosuppressive drug regimens. As post-transplant survival continues to improve, renal physicians will be faced with an increasing number of patients with renal failure requiring dialysis or kidney transplant.

In conclusion, as the prognosis for CF patients continues to improve it is likely that renal complications will become more common. There may also be more subtle primary abnormalities of renal function, perhaps with long term consequences, resulting from abnormal expression of CFTR in the kidneys. This is a potentially fruitful area for further research.

1.5 USE OF ANTIBIOTICS IN CF

1.5.1 Respiratory infections including *P. aeruginosa*

Although in the early stages of CF there may be few or no clinical abnormalities, bronchoscopy studies have documented respiratory infection and inflammation in well infants (Armstrong et al, 1995; Khan et al, 1995; Armstrong et al, 1996; Rosenfeld et al, 2001b).

Viral and bacterial infections with *Staphylococcus aureus* and *Haemophilus influenzae* predominate in younger patients. Chronic infection with *P. aeruginosa* used to be common in most patients by their late teens. This is no longer true. Lee

at al reported less than 20% of the paediatric and around 55% of the adult population attending the Leeds CF unit had chronic *P. aeruginosa* infection (Lee et al, 2004). The impact of this epidemiological change can be seen in the need to segregate cohorts into different clinics based on the colonising agent. Chronic infection can be prevented or at least substantially delayed by intensive treatment of the first *P. aeruginosa* isolate, using an eradication regimen of antibiotics, often comprising combinations of nebulised, oral and / or IV antibiotics (Vazquez et al, 1993; Frederiksen et al, 1997; Ratjen et al, 2001; Taccetti et al, 2005; Lebecque et al, 2006). The most effective regimen is still uncertain (Conway et al, 2003). It is critically important to offer this treatment to all patients because chronic *P. aeruginosa* infection results in worse clinical status, lower respiratory function, lower weight, height and BMI, more hospital admissions and increased treatment costs (Konstan et al, 1999; Emerson et al, 2002; Taccetti et al, 2005). In the majority of patients, chronic *P. aeruginosa* infection is with the mucoid form of the bacteria. This defends itself against innate immunity and phagocytosis by producing large amounts of a protective alginate-based biofilm (Lyczak et al, 2002). Alginate also protects *P. aeruginosa* against the activity of antibiotics (Aaron et al, 2002). The huge numbers of peripheral blood leucocytes which congregate in the lung in an attempt to kill these bacteria release powerful enzymes, directed at the pathogens but also damaging to the lung itself. These fuel a self-perpetuating inflammatory process in the lungs, the end result of which is destruction of lung tissue (Venaille et al, 1998). Following repeated respiratory tract infections it is the body's response to *P. aeruginosa* infection which is largely responsible for progressive lung damage (Heeckeren et al, 1997; Konstan and Berger, 1997).

1.5.2 Antibiotics and eradication of early or recurrent *P. aeruginosa* infection

Chronic *P. aeruginosa* infection is associated with the mucoid phenotype of *P. aeruginosa* (Lyczak et al, 2002), a more rapid decline in lung function and a worse prognosis. Strategies aimed at preventing or delaying progression from initial acquisition of *P. aeruginosa* to chronic infection are an essential part of CF

management and have proved highly effective. Recent data suggest that the window of opportunity for such strategies may be quite large (Li et al, 2005). Whilst acquisition of *P. aeruginosa* may occur quite early in life, the transition from the non-mucoid to the mucoid phenotype may take several years.

Evidence from several studies has shown that early administration of antibiotics, once colonisation with *P. aeruginosa* has been identified, significantly reduces the risk of chronic infection (Littlewood et al, 1985; Valerius et al, 1991; Frederiksen et al, 1997; Ratjen et al, 2001; Gibson et al, 2003b; Taccetti et al, 2005; Lebecque et al, 2006). A common feature of these trials is the use of aerosolised antibiotics. Conversely, trials using intravenous antibiotics for early eradication therapy have been disappointing (Steinkamp et al, 1989).

In the study by Valerius et al, 26 patients received PO ciprofloxacin plus nebulised colistin twice daily for 3 weeks or no treatment, in response to an initial isolation of *P. aeruginosa*. After 27 months of the trial significantly fewer patients who had received treatment were positive for *P. aeruginosa* (14% vs. 58%) (Valerius et al, 1991). This protocol was further refined by the Copenhagen group to increase the duration of treatment with oral ciprofloxacin and aerosolised colistin to three months. Follow up after three and a half years revealed that only 16% of treated patients developed chronic *P. aeruginosa* infection in comparison to 72% of untreated historical controls (Frederiksen et al, 1997). Ratjen et al gave 15 patients aerosolised tobramycin 80 mg daily for 12 months in response to initial isolation of *P. aeruginosa* (Ratjen et al, 2001). After one year follow up, 14/15 patients remained negative for *P. aeruginosa*. Gibson et al randomised 21 children under the age of six years who became positive for *P. aeruginosa* to receive 300 mg Tobramycin Solution for Inhalation (TOBI) or placebo twice daily for 28 days (Gibson et al, 2003b). At the end of treatment all eight children who received TOBI vs one of 13 who received placebo ($P<0.0001$) were negative for *P. aeruginosa*.

Early eradication therapy is believed to be a major reason for the increased survival of patients with CF, mainly as a result of reduction in the prevalence of chronic *P. aeruginosa* infection (Johansen et al, 2004; Lee et al, 2004).

There have been no studies comparing colistin with TOBI for early eradication of new *P. aeruginosa* isolates. While there is evidence for the efficacy of TOBI, the cheaper alternative, colistin, has been extensively used and has proved highly effective when combined with oral ciprofloxacin in preventing, or at least substantially delaying, chronic infection after the first *P. aeruginosa* isolate (Valerius et al, 1991; Frederiksen et al, 1997).

Eradication therapy is usually well tolerated. Absorption of nebulised tobramycin does not reach sufficient levels in the majority of patients to adversely affect renal function. A retrospective review of children with CF receiving inhaled gentamicin showed significantly raised N-acetyl-B-D glucosaminidase (NAG) activity indicating renal tubular damage compared to control children who had never received inhaled gentamicin or who had discontinued the drug at least three months previously. The long term clinical implications of these findings remain uncertain as urinary NAG activity returned to normal at the end of treatment.

There has been no evidence to suggest significant increases in antimicrobial resistance during eradication therapy, even after multiple repeat courses (HO et al, 2004). Aerosolised antibiotics and oral ciprofloxacin have been associated with an increased risk of colonisation with *Stenotrophomonas maltophilia* (Denton et al, 1996a), *Aspergillus* species (Bargon et al, 1999), and MRSA (Nadesalingam et al, 2005).

1.5.3 Antibiotics and treatment of chronic *P. aeruginosa* infection

1.5.3.1 Intravenous antibiotics

There are four main indications for IV antibiotic therapy:

i. *To eradicate P. aeruginosa infection*
   a. As an alternative to combined oral and nebulised antibiotic treatment e.g. when treating an infant or young child
   b. As a prelude to the standard oral and nebulised antibiotic regimen when a new *P. aeruginosa* culture is associated with new respiratory symptoms
   c. When a standard eradication regimen has failed

ii. *To treat a new cough that has not settled with the addition of an oral antibiotic*

If new respiratory symptoms do not settle after two different oral antibiotics, or if there are significant continuing symptoms after a single oral antibiotic course, treatment with oral antibiotics is recommended. It is important to remember that significant new infection may be present even when careful auscultation does not reveal new added sounds and in the absence of new radiological changes. There may only be a persistent or new cough. It is the general experience that the persistent cough dries up within a few days of starting IV antibiotics. The antibiotic prescribed will depend on previous respiratory cultures for the patient.

iii. *To treat a respiratory exacerbation*

Respiratory exacerbations are loosely defined in terms of more breathlessness, increased cough, change in sputum colour or volume, changes on chest radiographs, deteriorating respiratory function and loss of appetite and weight. An increased severity of two or more lower respiratory tract symptoms and a fall of 10% or more from baseline FEV1 or FVC were suggested to best reflect a new respiratory exacerbation

(Kanga et al, 1999; Dakin et al, 2001; Rosenfeld et al, 2001a). It is recommended that respiratory exacerbations are treated with two intravenous antibiotics to minimise the risk of antibiotic resistance developing (Cheng et al, 1996; Denton et al, 1996b). Combined therapy may also have an additive or synergistic effect. The duration of a course of IV therapy varies but is usually 2-3 weeks. Usually an aminoglycoside (e.g. gentamicin or tobramycin) is combined with a beta-lactam (e.g. ceftazidime, tazocin, meropenem or aztreonam) as recommended by the CF trust guidelines (Cystic Fibrosis Trust Antibiotic Group 2002). Colistin, a polymyxin, is often used as a second line agent, for example if there are concerns over allergy or bacterial aminoglycoside resistance (Conway et al, 1997; Littlewood et al, 2000). However, in certain centres such as ours, the emergence and spread of transmissible and multiresistant *P. aeruginosa* strains has dictated a greater utility for this old antibiotic (Ledson et al, 1998). The individual patient's history of antibiotic hypersensitivity reactions is taken into account when deciding treatment.

The initial choice of antibiotics still depends on the bacterial sensitivity pattern, although there is dispute over whether the laboratory-deduced sensitivities are of any relevance *in vivo*. The reliability of susceptibility testing of *P. aeruginosa* isolated from chronic infections in CF is uncertain. Foweraker et al showed that standard laboratory protocols often under-represented the complexity of resistance patterns and this contributed to widespread inter-laboratory variability in determining susceptibility patterns (Foweraker et al, 2005). Further evidence also suggests that the susceptibility pattern in chronic *P. aeruginosa* infection is poorly predictive of clinical response. As a result, an argument has been made to reduce the number of routine sensitivity tests done in CF clinics. Isolates of *P. aeruginosa* obtained from patients with CF and

chronic infection are only routinely tested if they have not been checked in the previous three months, or if obtained from a sample taken at the commencement of IV antibiotics, or if the patient is failing on their current antibiotic regimen (Etherington et al, 2008). Clinical improvement may reflect the drug's anti-inflammatory and/or anti-oxidant effects, or activity against *P. aeruginosa* virulence factors such as exotoxin A, total protease, elastase, phospholipase C, lipase and lecithinase, rather than its bactericidal effect (Adeboyeku et al, 1999; Ledson et al, 1999; Davey et al, 2000).

It is generally accepted that once-daily tobramycin is as effective and safe as thrice-daily administration (Master et al, 2001; Whitehead et al, 2002; Smyth et al, 2005; Smyth and Tan, 2006). Most patients find once-daily dosing more convenient. Trough drug levels should be monitored before the second dose and on day eight (although CF unit protocols vary) and should be <1 mg/l. In a small number of patients, toxicity may still occur despite trough levels of < 1 mg/l (Coulthard et al, 2007). Further monitoring may involve taking two blood samples at one and eight hours post dose.

Improvement during a course of IV treatment can be demonstrated by performing regular respiratory function tests and carefully assessing other symptoms and signs including body weight.

iv. As a routine three-monthly treatment for patients with chronic P. aeruginosa infection

This regimen was recommended by the Copenhagen group in 1989. They demonstrated a significantly better five year survival when patients with

chronic *P. aeruginosa* infection were treated with IV anti-pseudomonal antibiotics every three months irrespective of their clinical condition (Jensen et al, 1989; Frederiksen et al, 1996). This is now more debatable as the general picture of CF has changed and many CF physicians believe that only patients requiring this frequency of treatment to maintain their clinical stability should be so treated. For other patients, the risk of this "one prescription for all" approach in terms of inducing toxic effects on renal function, hearing and balance has not been fully quantified or understood but may outweigh the possible benefits of this treatment regimen (Ciofu et al, 1994; Elborn et al, 2000; Breen and Aswani, 2001). The physical status of patients with CF in 2009 is totally different to that in 1989 and most patients will keep well without resort to four IV antibiotic courses annually. Moreover, patients are living much longer and therefore the potential for serious adverse events from a lifetime of frequent antibiotic treatments is significantly increased (though not previously reported in a quantitative manner). A greater frequency of antibiotic use also increases the risk of patients developing hypersensitivity reactions to these medications (Koch et al, 1991; Burrows et al, 2007).

Finally, there is the cost of treatment to the hospital and health service and the extra costs incurred by hospitalisation for the patients and their carers.

1.5.3.2 Antibiotic treatment for multi-resistant organisms

Multiresistant isolates of *P. aeruginosa* are an increasing problem (Pitt et al, 2003). Innately resistant organisms such as BCC, *Stenotrophomonas maltophilia* and *Achromobacter xylosoxidans* are becoming more prevalent (Moore et al, 2001). MRSA is also a growing problem (Nadesalingam et al, 2005; Sawicki et al, 2009). These changing patterns probably result from greater patient longevity and

increased antibiotic use for acute exacerbations and maintenance care. The optimal treatment for these resistant bacteria is not known (Conway et al, 2003).

Multiresistant *P. aeruginosa* infection may be treated successfully by using two antibiotics with different mechanisms of action. In practice antibiotic choices have usually been made on a best-guess basis. It had been hoped that better directed therapy could have been achieved through the application of multiple combination bactericidal testing (MCBT) (Lang et al, 2000). This systematically tests isolates against different combinations of antibiotics to determine optimal sensitivity patterns. However, a follow up study showed that clinical outcomes were not significantly improved when using regimens selected on the basis of MCBT compared to standard susceptibility tests (Aaron et al, 2005).

The selection of antibiotics for pan-resistant bacteria is problematic. In practice, they should be treated with the antibiotics that by experience have produced the best clinical response in that individual patient. Combination antibiotic therapy is recommended (Cystic Fibrosis Trust Antibiotic Group 2002; Foweraker et al, 2005), but the choice of regimen should always be guided by the clinical response.

Resistance to colistin is rare and interest in this antibiotic has therefore increased in recent years (Ledson et al, 1998; Littlewood et al, 2000; Kasiakou et al, 2005). Some believe it is a valuable second line IV drug to be reserved for multiresistant *P. aeruginosa* (Conway et al, 1997; Conway et al, 2000; Falagas et al, 2009). Aerosol delivery of tobramycin solution for inhalation (TOBI) achieves high endobronchial concentrations that may overcome bacterial resistance as defined by standard laboratory criteria for IV therapy (Saiman et al, 1996; Lang et al, 2000). Its application in the treatment of CF acute pulmonary exacerbations is an attractive proposition, but whether this can be carried out successfully and safely is an

unanswered question. Mechanisms of antibiotic delivery (timing, dosage, infusion rate) should be reviewed to achieve optimal bactericidal effect. There is also a need to evaluate the efficacy of new antibiotic groups such as the macrolides.

1.5.3.3 Nebulised antibiotics

The advantages of nebulised antibiotic therapy for *P. aeruginosa* infection in CF have been recognised for over 30 years (Mearns, 1970). An antibiotic delivered directly to the site of infection should be most effective. The altered lung environment consequent on inadequate CFTR protein function may reduce drug accumulation by the bacteria, and aminoglycoside concentrations may be reduced by binding to the excess extracellular neutrophil DNA (Levy et al, 1983). Sputum concentrations 25 times greater than the minimum inhibitory concentration (MIC) may be necessary to achieve a bactericidal effect (Mendelman et al, 1985). These levels can not be reached by IV administration without unacceptable risks of systemic toxicity, but can be realised by inhalation of aerosolised antibiotics which, because of their minimal systemic absorption, are less likely to cause ototoxicity or nephrotoxicity (Smith et al, 1989).

Tobramycin crosses the placenta and there is a theoretical risk of damage to the VIII cranial nerve and of nephrotoxicity. Avoidance of parenteral administration is recommended during pregnancy but the risks from nebulised administration are much less. A decision whether or not to continue nebulised antibiotic treatment during pregnancy should be made on an individual basis and consultation with the patient. The minimal theoretical risk to the baby of continued treatment should be weighed against the risks to the mother's health of stopping treatment.

Clinical indications for nebulised antibiotics:

i. *Delay or prevention of chronic P. aeruginosa infection (see above)*

ii. *Prevention of clinical deterioration in patients chronically infected with P. aeruginosa*

Regular nebulised antibiotics reduce the rate of decline of respiratory function in patients chronically infected with *P. aeruginosa*. In 1981 Hodson et al. compared six months of treatment with twice daily nebulised gentamicin (80mg) and carbenicillin (1gm) vs. placebo (Hodson et al, 1981). Treated patients had significantly improved respiratory function and a non-significant trend towards fewer hospital admissions. Follow up studies confirmed the benefits of treatment for chronic *P. aeruginosa* infection: improved lung function, a slower decline in lung function, fewer hospital admissions, better clinical scores and weight, and decreased *P. aeruginosa* density and production of virulence factors (Conway, 1999). There was no renal toxicity, ototoxicity or increase in bacterial resistance (Touw et al, 1995; Mukhopadhyay et al, 1996).

Low systemic and high local concentrations of colistin after nebulised delivery (Ratjen et al, 2006), and a randomised double blind study of TOBI (Ramsey et al, 1999) support their use in patients with chronic *P. aeruginosa* infection. In the TOBI study, the first cycle of treatment produced a 12% increase in FEV1 which was maintained through the study. There was also a significant fall in colony forming units per gram of sputum, and patients required fewer IV antibiotic treatments. Sputum drug concentrations greater than 25 times the MIC value were seen in 95% of patients. Adolescent patients responded particularly well with 14% improvement in FEV1 compared 1.8% for controls (Moss, 2002). Increasing *P. aeruginosa* tobramycin resistance was not associated with reduced clinical efficacy (Ramsey et al, 1999). There was no increased

isolation of intrinsically tobramycin-resistant microorganisms (Burns et al, 1999).

Colistin resistance is rare (Denton et al, 2002; Govan, 2002; Landman et al, 2008) and there is general consensus that sensitivity returns when colistin inhalation and IV use are suspended for six months (Landman et al, 2008). A comparative study of twice daily nebulised TOBI (300 mg) and nebulised colistin (1 megaunit), at present the only antibiotics licensed in the UK for nebulisation in CF, showed that both treatments reduced the bacterial content of the sputum significantly. FEV1 increased by 6.7% with TOBI and 0.37% with colistin (Govan, 2002; Hodson et al, 2002).

The Cochrane review found insufficient evidence to claim superiority for either TOBI or colistin. Eleven trials met the inclusion criteria. The review concluded that nebulised antibiotic treatment improves lung function and reduces the frequency of pulmonary exacerbations. There was no evidence of clinically important adverse events (Ryan et al, 2003).

Long term treatment with nebulised antibiotics is effective with patients having fewer hospital admissions and IV antibiotic use and better preservation of respiratory function (Moss, 2001). Long term treatment is generally safe but patients show an unpredictable range of systemic antibiotic absorption. The possibility of toxic drug levels resulting from nebulised antibiotic delivery should be remembered. Acute renal failure after one week of TOBI and concurrent ciprofloxacin, and reversible vestibular dysfunction due to inhaled tobramycin in a patient receiving haemodialysis have been reported (Hoffmann et al, 2002; Edson et al,

2004). Following inhaled gentamicin children showed significantly raised, but reversible, urinary NAG activity indicating renal tubular damage, compared to control children who had never received gentamicin inhalation or who had discontinued the drug at least three months previously. There was a positive correlation between NAG levels and cumulative antibiotic dose (Ring et al, 1998).

iii. Other uses of nebulised antibiotics

a. Acute respiratory exacerbations

There are no trials showing that nebulised antibiotics are as effective as IV antibiotics for treating infective exacerbations, or that they are useful adjuncts to IV therapy (Stephens et al, 1983; Schaad et al, 1987; Semsarian, 1990).

Nonetheless, clinicians may wish to use TOBI for the treatment of exacerbations associated with multiresistant *P. aeruginosa* because of the high endobronchial antibiotic levels achieved. The high sputum drug concentrations may render the usual laboratory breakpoints meaningless (Saiman et al, 1996; Lang et al, 2000).

b. To prevent *P. aeruginosa* infection

Twice daily inhaled gentamicin in a small group of very young children prevented chronic infection for a mean of 78 months (Heinzl et al, 2002). Regular use of nebulised TOBI, colistin, injectable forms of tobramycin or amikacin are associated with a chronic *P. aeruginosa* infection rate of <3% in Belgian children (Lebecque et al, 2006). There are however important negative effects to be considered before adopting this proactive approach. These include the increased risks of bacterial resistance, the risk of emergence of fungal organisms, the potential toxicity of

treatment and the impact on daily life of long-term nebulised antibiotic treatments. Chronic infection can also be prevented in the majority of children with less invasive protocols aimed at eradicating new *P. aeruginosa* infection.

c. Treatment of nontuberculous mycobacterial infection

Treatment should be considered for patients who are smear positive, and for those with persistent positive cultures and symptoms despite routine antibiotic treatment.

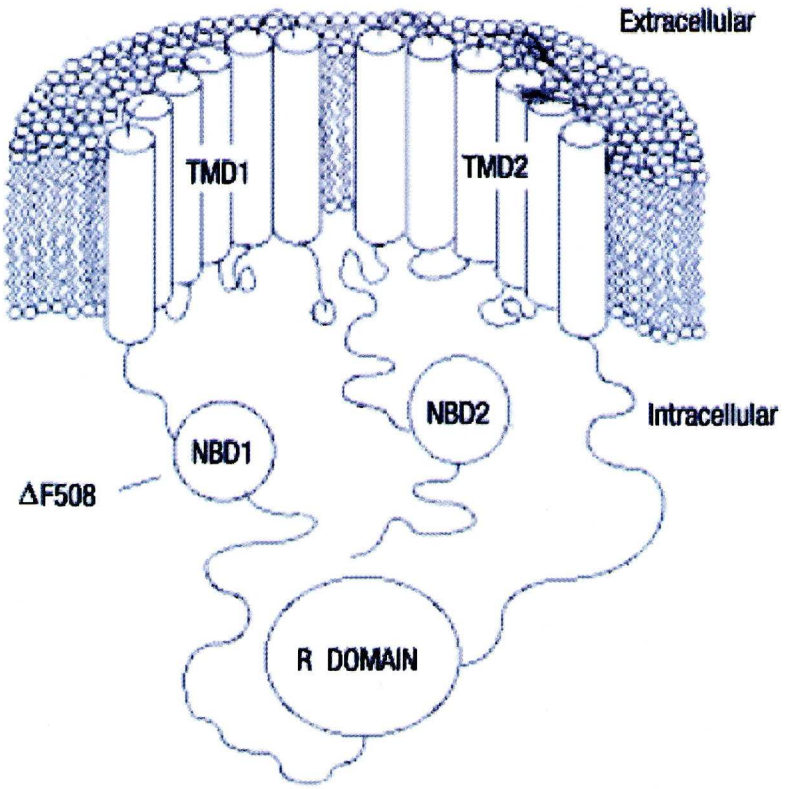
Nebulised amikacin is part of the complex and long term antibiotic regimens for the treatment of the rapid growers, such as *M. abscessus* (Cullen et al, 2000). There is no evidence base for dosage but 500mg bd is recommended. This may need reducing to 250mg bd in younger children.

d. Nebulised vancomyin for the treatment of MRSA

1.5.3.4 Long term oral azithromycin (see above under tubulointerstitial nephritis)

1.6 FIGURES

Figure 1.6.1 - Predicted two-dimensional structure of the CFTR protein



The CFTR domains shown are: TMD1 and TMD2-transmembrane spanning domains 1 and 2, respectively; R domain-regulatory domain; NBD1 and NBD2-nucleotide binding domains 1 and 2, respectively. The most common mutation, ΔF508, is found in NBD1.

CHAPTER TWO

Aim and outline of this study

2.1 AIM OF THE PRESENT STUDY

It is obvious from the literature review presented in chapter one that CF patients are exposed to a high burden of potentially nephrotoxic therapy to manage both their day-to-day stability and as rescue treatment for pulmonary exacerbations. The aim of this study was to search for and quantify any resulting impairment in their renal reserve, evaluate different methods of assessing renal function in this patient population, and examine in prospective trials measures aimed at kidney protection in the face of ongoing obligatory need for nephrotoxins.

2.2 OUTLINE OF THE PRESENT STUDY

In Chapter 4, I describe a prospective cross sectional study in which creatinine clearance was measured in stable adult CF patients attending our regional outpatient clinic. The study aimed to identify those with impaired renal function and examine potential association with prior exposure to different classes of nephrotoxic antibiotics.

As the lower limit of normal GFR is set at 80 ml/min, impaired renal function encompasses a wide range of reduced GFR values. The study in chapter 4 identified many patients with only mild renal dysfunction. However, the relevance of this finding to clinical practice is highlighted by a case series of acute renal failure detailed in chapter 5.

So, what can we do about it?

Before changes to our antibiotic policies could be proposed in order to extend the renal reserve of our patient, a key question to address is how best to assess renal function in CF. This includes:

1. Means of *screening* for background levels of reduced GFR in the clinic population
2. Methods to quantify the magnitude of *acute renal toxicity* related to a current course of antibiotics

For the former, chapter 6 summarises a comparison of the performance of 8 formulae developed for estimation of GFR *vs* direct measurement of CCl from 24 hour urine collections. For the latter, urine proteins and enzymes are used as early biomarkers of renal tubulotoxicity. These are employed in chapter 7 to examine possible mechanisms of cumulative tubular injury with repeated exposure to nephrotoxic antibiotics.

Chapters 8 and 9 subsequently apply these sensitive urinary biomarkers in prospective cross over trials, wherein alternative potentially nephroprotective antibiotic strategies are compared with standard therapy for acute pulmonary exacerbations. The studies presented assess efficacy and safety of the different antibiotic combinations including their impact on GFR as well as tubular structural integrity and function.

Some of the conclusions reached in the above studies have led to a natural change in our antibiotic policies and these, along with other general recommendations aimed at protecting renal function in C F patients, are discussed in the final chapter. Proposals for future research in this field are also presented.

CHAPTER THREE

General laboratory methods

3.1 SPIROMETRY

Lung function was measured by qualified lung function physiologists (BSc in Clinical Physiology and the Association for Respiratory Technology and Physiology professional qualifications) on a standard volumetric spirometer (Vitalograph, Ennis, Ireland) with the patient in erect position. Tests were performed to standards of the joint American Thoracic Society/European Respiratory Society guidelines (Miller et al, 2005). Flow was calibrated twice daily using a 3-litre syringe with accepted tolerance of +/- 3%. The best of three measurements was recorded; forced expired volume in one second (FEV1) and forced vital capacity (FVC) were noted.

3.2 SERUM AND URINE CREATININE

Serum and urine creatinine concentrations were measured according to the Jaffe reaction, using a standard alkaline picrate assay through a Roche clinical chemistry autoanalyzer.

3.3 MEASURED CREATININE CLEARANCE (mCCl)

This was assessed in every patient by comparing the urinary creatinine (UCr) cleared over 24 hr with a paired spot serum creatinine (SCr) obtained after an 8 hour fast at the end of each 24-hr collection period.

The renal clearance of endogenous creatinine was determined by the formula:

$$mCCl = UCr * urine\ volume/SCr$$

and expressed as ml/min adjusted, where appropriate, for body surface area ($CCl/1.73\ m^2$).

3.4 SERUM TOBRAMYCIN

Serum tobramycin concentrations were measured in the hospital clinical biochemistry laboratory using TDxFLx assay (Abbott Laboratories; lower limit of quantitiation 0.2 mg/l).

3.5 URINE PROTEINS AND ENZYMES

In the studies where proteinuria and enzymuria were measured, urine was collected in standard containers, kept in an opaque sheath on ice at the patient's bed side. For each 24 hour urine collection, the total urine volume was recorded and an aliquot of 20 ml frozen at -80°C until assayed.

3.5.1 Urine total protein and albumin

Total protein in urine was measured by a turbidimetric method in a Roche Clinical Chemistry Analyser (Wako, Japan). The sample is preincubated in an alkaline solution of sodium hydroxide, 530 mmol/l, containing EDTA-Na at 74 mmol/l, which denatures the protein and eliminates interference from Mg^{2+} ions. Benzothonium chloride is then added, producing a turbidity that is read at 505 nm. The lower detection limit (the lowest measurable analyte level that can be distinguished from zero) for this method is 40 mg/l. The local laboratory reference value is < 150 mg/l or < 200 mg/24 hours (Junge et al, 2006).

Urinary albumin was quantified by by an immunoturbidimetric assay in a Roche analyser. This uses sheep polyclonal anti-human albumin antibodies in TRIS buffer: 100 mmol/l, at pH 7.2 and 25°C. The lower detection limit is 3 mg/l and the local laboratory reference is < 2.5 mg albumin/mmol creatinine for males and < 3.5 mg/mmol for females in random samples and < 30 mg/24 hour urine collections.

3.5.2 Urinary N-acetyl-β-D glucosaminidase (NAG)

NAG (Yuen et al, 1982; Marchewka and Dlugosz, 1998) was measured using a commercially available assay kit [PPR Diagnostics, London] optimised for use in 96-well plate format. 8μl of urine was added to 120μl of substrate (2-methoxy-4-(2'-nitrovinyl)-phenyl 2-acetamido-2deoxy-β-D-glucopyranoside) in a citric acid

/phosphate buffer and incubated for 30 minutes at 37°C. Production of the coloured product 2-methoxy-4-(2'-nitrovinyl)-phenol by NAG hydrolysis was measured at 505nM after addition of 40μl of KCl/KH_2PO_4 buffer at pH 9.5. Calibration was achieved by reference to a standard curve of bovine kidney NAG of defined activities. The effects of varied urine concentration were minimised by expressing NAG level as a ratio of urine creatinine. Results are reported as units/mmol creatinine.

3.5.3 Urinary alanine aminopeptidase (AAP)

AAP (expressed as units/mmol creatinine) was measured using a modification of a procedure described by Jung and Scholz (Jung and Scholz, 1980). Urine samples were prepared using PD10 desalting columns [Amersham Biosciences, Bucks, UK]. In 96 well microplates, 30μl of desalted urine was added to 150μl of 59mM tris/HCL buffer (pH 7.8) and allowed to equilibrate at 37°C for 15 minutes. The reaction was started with the addition of 30μL of substrate alanine-4-nitroanilide hydrochloride (26mM in tris buffer). The reaction was monitored over 5 minutes and the change in absorbance at 410nm calibrated by reference to a standard curve of known concentration of p-nitroaniline product.

3.5.4 Urinary β_2-microglobulin (β_2M)

β_2M (Herber, 1984) (expressed as mcg/mmol creatinine) was measured from spot urines collected at 14:00 hours. Samples were immediately buffered to a pH of 7.0 and frozen at -80°C until analysed with a solid immunoradiometric assay [Euro/DPC, Llanberis, UK].

For all urine enzymes and proteins, the effects of varied urine concentration were minimised by expressing levels of the substance of interest as a ratio of urine creatinine.

CHAPTER FOUR

Renal impairment in CF patients due to repeated intravenous aminoglycoside use

4.1 INTRODUCTION

Although cystic fibrosis (CF) is a multisystem disorder, the only abnormalities traditionally associated with the renal tract are an increased incidence of nephrolithiasis (Hoppe et al, 1998; Perez-Brayfield et al, 2002) and more recently, mechanical urological problems associated with coughing (Orr et al, 2001). Several cases of acute renal failure were noted in patients attending the adult CF unit in Liverpool which were associated with the concurrent use of potentially nephrotoxic antibiotics, and this was also reported elsewhere (Kovesi et al, 1998; Drew et al, 2002). The increasing prevalence of antibiotic multiresistance in *P. aeruginosa* strains in CF patients (Pitt et al, 2003; Spencker et al, 2003), and in particular that associated with epidemic strains (Cheng et al, 1996; Jones et al, 2001), may increase the requirements for nephrotoxic antibiotics. Indeed, the most prevalent epidemic strain in the United Kingdom (UK) (the Liverpool epidemic strain; LES) (Scott and Pitt, 2003) is common in my CF unit and is frequently sensitive only to tobramycin and colistin sulphomethate (Mirakhur et al, 2003), both of which are potentially nephrotoxic (Price and Graham, 1970; Bennett, 1983). Although other workers reported acute changes in markers of renal tubular function during courses of intravenous (IV) aminoglycoside therapy (Reed et al, 1981; Steinkamp et al, 1986), I was not aware of any studies documenting the level, if any, of background renal impairment in CF patients. I hypothesized that cumulative IV aminoglycoside and colistin exposure may adversely affect creatinine clearance, a measure of glomerular filtration rate (GFR) and hence renal function. To explore this further, I prospectively recorded GFR in a group of adult CF patients who had no history of renal problems, using both measured (24-hr urine collection) and calculated (formula of Cockcroft and Gault (Cockcroft and Gault, 1976)) creatinine clearance, and compared it with their lifetime use of these potentially nephrotoxic antipseudomonal antibiotics.

4.2 PATIENTS AND METHODS

4.2.1 Study population

Eighty consecutive CF outpatients chronically infected by *P. aeruginosa* (defined as three or more positive sputum cultures within a 6-month period) and who were in a stable clinical state formed the study population [mean age: 24.2 years (SD 7.6; range 16–56), mean forced expired volume in 1 sec (FEV1) % predicted: 63 (SD 24.6; range 16–117), and mean body mass index (BMI) 20.9 (SD 3.3; range 14–29.5), 40 male]. Patients co-infected with the *Burkholderia cepacia* complex were excluded. Twenty-three had CF-related diabetes mellitus (CFRDM), and 11 had liver disease. No patient had undergone transplantation or was taking immunosuppressive therapy known to cause renal disease. All patients gave informed consent, and the local ethics committee approved the study. All patients had previously required courses of intravenous antibiotics, and all had been treated with IV aminoglycosides and/or colistin. Colistin was given at a dose of 2 megaunits tds in the adult population and at 75,000 unit/kg/day in 3 divided doses in childhood. According to local protocols, aminoglycoside levels had been measured and the dose adjusted at the fourth administration to achieve a trough level of <1.0 mg/l and a peak level of 6–10 mg/l. No patient had any previous history of renal problems, including acute drug-related nephrotoxicity. In addition, 49 patients (61%) had a recent negative urinalysis, and all had normal renal ultrasound scans at annual screening.

4.2.2 Assessment of renal function

4.2.2.1 Measured Creatinine Clearance (mCCl)

See chapter 3 (General laboratory methods) for measurements of serum and urine creatinine and subsequent calculation of mCCl. A total of 163 24-hr urine collections were obtained (mean: 2 collections per patient; range: 1–5).

Twenty-four-hour urine collections were validated according to the method of Thakur et al (Thakur et al, 1997). Briefly, the mean urinary creatinines for men

(0.193 (SD: 0.047) mmol/kg) and women (0.138 (SD: 0.043) mmol/kg) were calculated separately, and all 24-hr urine collections that had values within 1 SD of these respective means were considered valid and were included for further evaluation. Using this validation method, 135 collections (83%) were suitable for further study. Each patient had at least one valid specimen, and in patients where more than one 24-hr collection was valid, the best (highest) measured creatinine clearance (mCCl) was chosen for analysis.

4.2.2.2 Estimated Creatinine Clearance (eCCl)

This was calculated using the formula of Cockcroft and Gault (Cockcroft and Gault, 1976) where serum creatinine is adjusted for age, sex, and body mass to estimate the creatinine clearance:

$$eCCl = (140 - age) * weight\ [kg]/72 * SCr\ [mg/dl] * (0.85\ for\ females)$$

The minimum value for normal creatinine clearance in our laboratory is set at 80 ml/min/1.73 m^2.

4.2.3 Assessment of aminoglycoside and colistin use

Hospital and central home care records were examined to ascertain the total number of IV courses of aminoglycosides and colistin each patient received up to the point of urine collection. To ensure that assessment of lifetime use of these therapies was complete, records from referring paediatric clinics to the Liverpool adult CF unit were retrieved and examined for data regarding IV antibiotics.

In line with the UK CF Trust guidelines (Cystic Fibrosis Trust Antibiotic Group 2002) a minimum of two antipseudomonal antibiotics were used to treat pulmonary exacerbations. In the Liverpool adult CF unit, IV quinolones are not administered and oral ciprofloxacin is reserved for outpatient treatment. Nebulised antibiotics are discontinued during IV therapy. Table 4.7.1 summarizes the number of courses of different combinations of antipseudomonal agents used intravenously. The median length of IV antibiotic courses was 14 days; the range was 7–49.

4.2.4 Statistical methods

The relationship between renal function and IV antibiotic use was examined with a multiple linear regression model, with CCl as the dependent variable and the number of IV courses of aminoglycoside + β-lactam, colistin + β-lactam, and aminoglycoside + colistin as the independent variables. Age, CFRDM, BMI, supplemental feeding, FEV1 (% predicted), and the use of azithromycin and nonsteroidal anti-inflammatory agents (NSAIDs) were considered potential confounders.

Pearson's correlation was calculated for the comparison of mCCl and eCCl. Proportions were compared with a χ^2 test. $P \leq 0.05$ was considered statistically significant. Analysis was carried out with StatsDirect, version 2.3.

4.3 RESULTS

All patients had blood urea and serum creatinine values within normal range (mean 4.6 mmol/l (SD: 1.7) and 84.2 (SD: 16.7) umol/l, respectively). The mean creatinine clearance using either technique was within normal range (mCCl: 84.6 ml/min/1.73 m^2 (SD: 25.6); eCCl: 89.5 (SD: 23.2) ml/min) (P=NS). However, 34 patients (42.5%) had a mCCl<80 ml/min/1.73 m^2 (mean: 61.8 (SD: 12.3) ml/min/1.73 m^2), indicating a degree of renal impairment. Similarly, the eCCl was abnormal in 25 patients (31.3%) (mean: 64.4 (SD: 16.4) ml/min). The two measures of renal function correlated significantly (r=0.59, P<0.001; Figure 4.8.1). The degree of diminished creatinine clearance in affected patients is shown in Table 4.7.2.

There was an inverse relationship between creatinine clearance and the total number of IV aminoglycoside and IV colistin courses (Figure 4.8.2). Compared with eCCl, 24-hr urine based measurements displayed a stronger correlation with IV antibiotic use (eCCl: r= -0.40, P<0.0001, vs. mCCl, r= -0.65, P<0.00001). In the multiple regression analysis model, increasing lifetime use of aminoglycosides administered with nonnephrotoxic antibiotics displayed a significant association with declining creatinine clearance. However, when administered with

nonnephrotoxic antibiotics alone, colistin was not associated with loss of renal function *per se* but enhanced the apparent nephrotoxicity of aminoglycosides when the two antibiotics were coadministered (Table 4.7.3). In this regression model, the described interactions between CCl and antibiotic use were independent of patient age, CFRDM status, BMI, supplemental feeding, FEV1 (% predicted), and azithromycin and NSAIDs use (P=NS, for all interactions).

The prevalence of diabetes did not differ between those with and without renal impairment (11/34 vs. 12/46, χ^2 =0.37, P=0.54), nor was there a difference in serum urea levels (mean: 4.80 (SD: 1.96) mmol/l vs. 4.38 (SD: 1.37) mmol/l, P=0.35).

4.4 DISCUSSION

Traditional indices of assessing renal function, including urinalysis and measurement of blood urea and serum creatinine, are insensitive and unreliable markers of early renal disease. Excretory renal function depends on the glomerular filtration rate (GFR), for which in healthy subjects there is an enormous reserve capacity, and serum urea and creatinine do not rise above normal range until at least 60% of GFR is lost (Baker, 2002). Indeed, using these parameters, gross nephrotoxicity was not detected in any of the studied patients despite 30–40% of the cohort having impaired renal function. This is in keeping with previous data from smaller studies that used [^{125}I]iothalamate clearance, proteinuria and/or enzymuria as markers of renal function (Reed et al, 1981; Steinkamp et al, 1986; Godson et al, 1988). Hence, when the serum urea and creatinine are within normal range, the measurement of GFR is essential to assess renal function. In normal individuals, renal removal of endogenous creatinine produced by muscle cell turnover is a reasonably accurate measure of GFR, and a comparison of serum and urine creatinine (creatinine clearance) is readily available in clinical practice (Bannister and Field, 1996). I therefore used this as a measure of renal function in my patients. However, poor patient compliance can lead to collection errors, and such a method may be difficult in a paediatric setting. In this study, I aimed to use more than one urine collection per patient to reduce compliance errors.

Furthermore, repeated samples were validated according to the method described by Thakur et al (Thakur et al, 1997) to ensure that only correctly collected specimens were included. One is therefore confident that this technique reflected the measured creatinine clearance as accurately as practically possible. Nevertheless, it has been suggested that when muscle creatinine output is reduced (e.g., low muscle mass, malnutrition), serum and therefore urinary creatinine will be low, such that this method may underestimate GFR and give a false indication of renal disease (Tan et al, 2003). CF patients may fall into the group who have low muscle mass.

In order to address this, several formulae were created to assess CCl by comparing serum creatinine with the predicted creatinine production, based on an estimation of muscle mass. The most widely used of these is that produced by Cockcroft and Gault (Cockcroft and Gault, 1976), and this was validated in several patient groups (Sampson and Drury, 1992; Thakur et al, 1997). However, the Cockcroft-Gault formula should be used with caution, since it was generated not to estimate GFR but to predict creatinine production from serum creatinine by factoring in weight, sex, and age. Using creatinine production as an estimate of urinary creatinine clearance assumes that the renal handling of creatinine is both normal and constant. This does not apply to patients with damaged renal function and can result in a gross overestimation of GFR, especially when the true GFR is <80 ml/min (Thakur et al, 1997; Alcanatra et al, 1998). Indeed, it was shown that the Cockcroft-Gault formula will overestimate GFR by 71% in patients with reduced renal clearance (Guasch et al, 1996). Although its application in CF patients was recently recommended (Tan et al, 2003), its use has not been proven in this condition, and the evidence for its validity in low muscle mass patients is also contradictory (Mirahmadi et al, 1983; Thakur et al, 1997). Nevertheless, in order to circumvent possible problems associated with either method, I applied both 24-hr urine collections and the Cockcroft-Gault formula to assess renal function in this patient group. I also ensured that all patients were clinically stable, such that the potential confounding effects of acute illness or a catabolic state would not falsely lower their

creatinine clearance. In keeping with this, the two methods correlated well, and adjusting the regression analysis for BMI did not alter the results. One accepts that an effect of renal nutrition on GFR cannot be entirely excluded, since the lack of difference in serum urea levels between those with and without renal impairment suggests that one of the other factors that influence urea (such as the amount or type of dietary protein) may be active.

I also adjusted the regressional analysis for most potentially confounding factors. Although ciprofloxacin was shown to cause acute renal failure, I did not include this in the regressional analysis model, since its nephrotoxicity is idiosyncratic (Lomaestro, 2000) and not related to cumulative exposure. Furthermore, in the UK, community physicians often give such therapy, making assessment of lifetime exposure impossible.

Nevertheless, using these techniques, this is the first report demonstrating a high prevalence of abnormal renal function in adult CF patients: 42% by mCCl and 31% by the Cockroft-Gault formula had a creatinine clearance below the normal range. Although the threshold I used for this (80 ml/min/1.73 m^2) is derived from non-CF individuals, most published data suggest that GFR in CF patients is similar to age-matched controls (Assael et al, 1986; Rey et al, 1998) and I therefore believe that this value is valid in CF.

Furthermore, I was able to demonstrate a strong relationship between degree of renal impairment and the use of nephrotoxic antibiotics, which appeared to be associated primarily with the use of aminoglycosides. Nephrotoxicity is a dose-limiting feature of aminoglycosides, which have a narrow margin of safety, with toxic levels close to the therapeutic range (Samaniego-Picota and Whelton, 1996). Renal impairment is associated not only with excess levels of aminoglycosides but also with cumulative lifetime dose (Pedersen et al, 1987). Acute aminoglycoside-induced renal injury is believed to be at the proximal tubule, with resultant leak of serum electrolytes (Akbar et al, 1999) and the appearance of cell-bound enzymes

and protein in the urine (Reed et al, 1981; Steinkamp et al, 1986; Godson et al, 1988) reflecting early structural and/or functional changes. In keeping with this, acute tubular necrosis was documented at renal biopsy in CF patients with acute renal failure caused by aminoglycoside therapy (Drew et al, 2002). The results of this relatively large cross sectional study also show a further adverse effect on glomerular filtration with long-term aminoglycoside use.

Although case reports of acute renal failure in CF (Kovesi et al, 1998; Drew et al, 2002) implicated predominantly gentamicin, tobramycin and gentamicin are thought to be similarly nephrotoxic (Burkle, 1986) and I therefore did not attempt to separate the effects of these two agents. Furthermore, a recent study suggested that aminoglycosides may be less nephrotoxic when given once daily (Smyth et al, 2005). However, all patients in the Liverpool adult CF unit have experienced variable dosing regimens over time, and I was therefore unable to differentiate this effect in the study group.

In this study, the nephrotoxic effect of aminoglycosides was potentiated by the coadministration of colistin, while the use of colistin without aminoglycosides but in conjunction with other antibiotics did not appear to be nephrotoxic. Interestingly, although the development of severe (sometimes irreversible) renal failure was one of the reasons the polymyxin family of antibiotics fell out of favour in the early 1970s, the doses used were very high (Price and Graham, 1970). The study presented herein suggests that colistin used in moderate doses is not nephrotoxic, but must only be coadministered with aminoglycosides with caution. Unfortunately, many CF patients in Liverpool are colonized by a highly multiresistant strain of *P. aeruginosa* (the Liverpool epidemic strain; LES) that is frequently sensitive only to tobramycin and colistin (Mirakhur et al, 2003). Thus, many of these patients will have been coadministered courses of these two antibiotics repeatedly for good clinical reasons.

The emergence of these multiresistant *P. Aeruginosa* strains in CF has resulted in the repeated use of a limited number of antibiotics to which the organisms are sensitive, increasing the risk of toxic effects. Colonization by multiresistant strains may occur in two ways. First, the repeated use of antibiotics will select out resistant strains, and the practice of routine 3-monthly IV antibiotic therapy in some paediatric centres may exacerbate this (Ciofu et al, 1994). Furthermore, compared with conventional "on demand" treatment, this practice confers no advantage in pulmonary function (Elborn et al, 2000; Breen and Aswani, 2001) and may be associated with higher mortality (Elborn et al, 2000). Indeed, many patients in the present study had received such prophylactic IV therapy in childhood before transfer to the adult CF unit after adolescence. Any apparent short-term benefit of such a policy must therefore be weighed against the possibility of renal damage in the long term. The adult CF physicians in Liverpool are now unable to use these powerful antibiotics in some of their patients because of their compromised renal function, and have had to modify the dosage regimens in other patients with careful monitoring of parameters of renal function. Second, epidemic *P. aeruginosa* strains tend to be multiresistant (Cheng et al, 1996; Jones et al, 2001) and there is evidence that such strains are becoming more prevalent in CF clinics (Armstrong et al, 2002; Scott and Pitt, 2003) possibly due to a lack of effective segregation policies.

In summary, this study showed that cumulative aminoglycoside use in CF patients is associated with long-term renal impairment. Furthermore, although this effect is potentiated by the coadministration of IV colistin, this therapy on its own in moderate doses does not appear to be nephrotoxic. Further research is required to define the mechanism of renal damage due to these therapies, and to explore potential renoprotective strategies. We recommend that all CF patients who require such antibiotic therapy undergo surveillance at least annually for renal impairment. In the paediatric sector, this may require the use of formulae to calculate creatinine clearance, while in adult patients, measured creatinine clearance at the time of annual screening may be more appropriate.

This work has been published

Al-Aloul M, Miller H, Alapati S, Stockton PA, Ledson MJ, Walshaw MJ. Renal impairment in cystic fibrosis patients due to repeated intravenous aminoglycoside use. *Pediatr Pulmonol*. 2005; 39(1):15-20

4.7 TABLES

Table 4.7.1 - Number of courses of different combinations of IV antipseudomonal antibiotics per patient

	Aminoglycoside + β-lactam	Colomycin+ β-lactam	Aminoglycoside + colomycin coadministered	Total courses including aminoglycoside and/or colomycin
Median	12	9	6	40
IQR	18	13	12.5	51.5
Mean	13.9	11	8.7	41.7
SD	14.8	11.1	10.1	34
Range	0-61	0-47	0-53	1-130

TABLE 4.7.2 - Degree of diminished creatinine clearance

Creatinine clearance	Measured (mCCl) (ml/min/1.73 m^2), number of patients	Calculated (eCCl) (ml/min), Number of patients
<30	4	2
30-39	1	0
40-49	4	2
50-59	8	4
60-69	13	4
70-79	4	13
Total <80	34	25

TABLE 4.7.3 - Correlation between renal function and lifetime IV antibiotic use [r (P Value)]

	mCCl	eCCl
IV aminoglycoside + β-lactam	-0.35 (P=0.0018)	-0.32 (P=0.0055)
IV colomycin + β-lactam	0.02 (P=0.83)	0.18 (P=0.15)
IVaminoglycoside + colomycin	-0.51 (P<0.0001)	-0.42 (P<0.0002)

4.8 FIGURES

Figure 4.8.1

Relationship between mCCl and eCCl. r=0.59, P<0.001

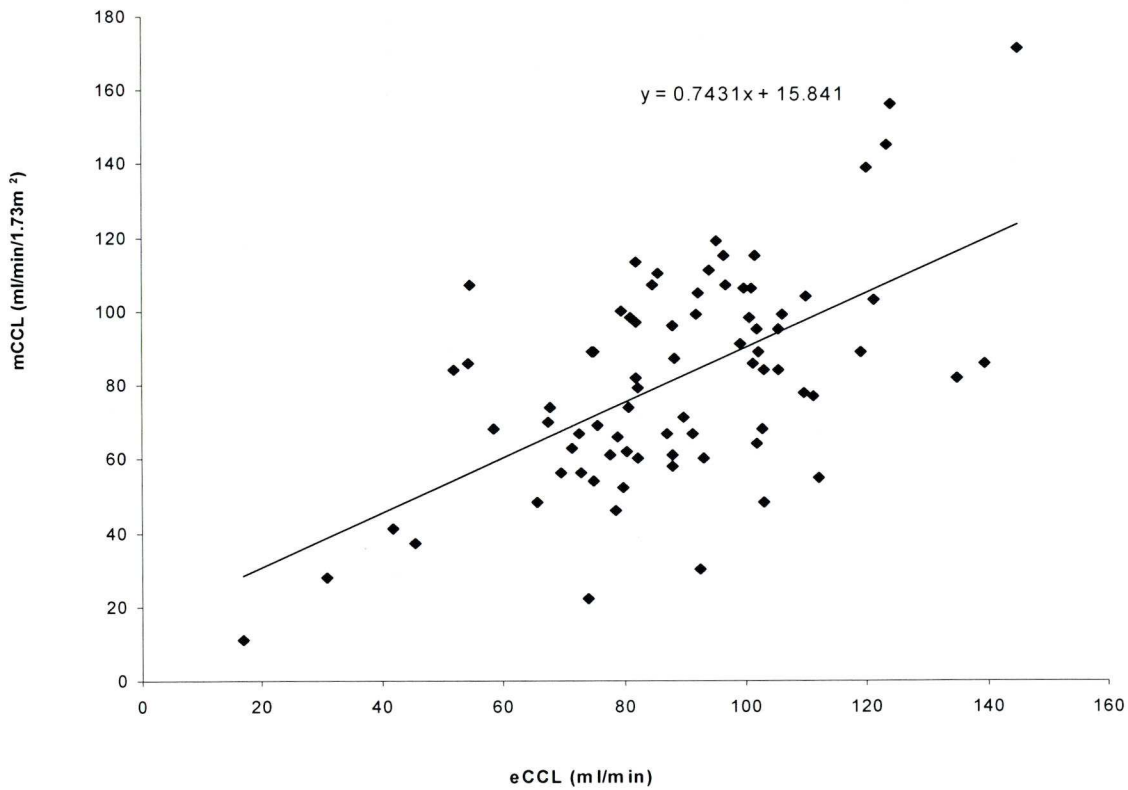
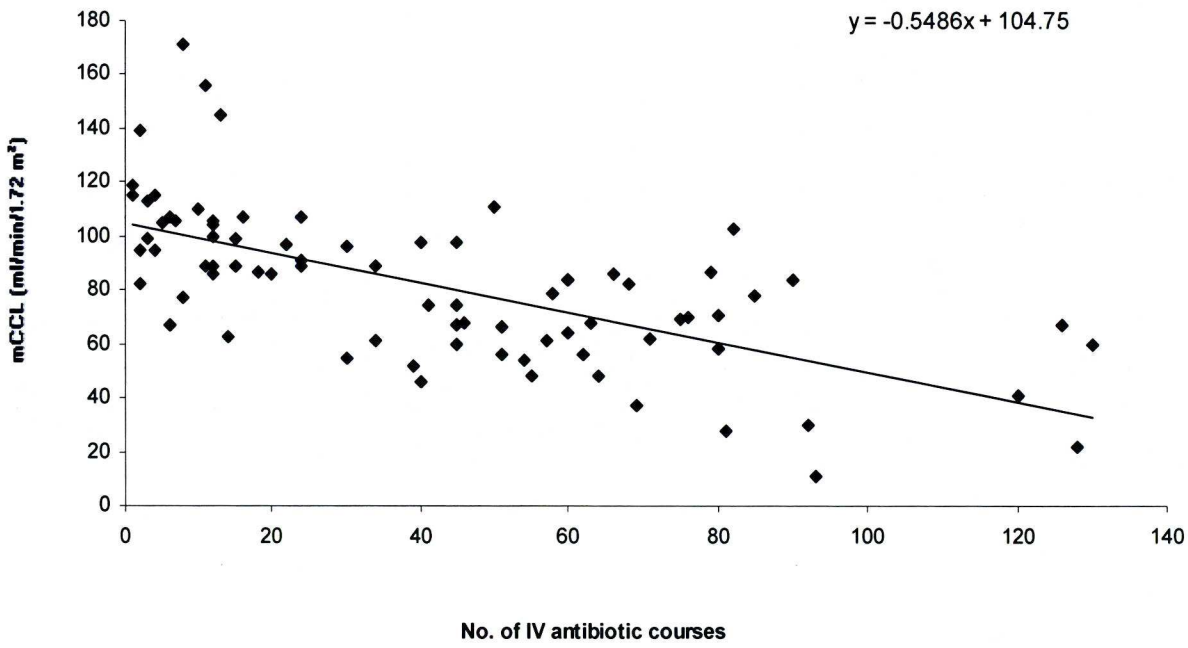


Figure 4.8.2

Correlation between renal function (mCCl) and lifetime use of IV nephrotoxic antibiotics (courses containing aminoglycosides and/or colistin).

r =0.65, P<0.00001



CHAPTER FIVE

Acute renal failure in CF patients chronically infected by the Liverpool epidemic *P. aeruginosa* strain

5.1 INTRODUCTION

Cystic fibrosis patients chronically infected with *P. aeruginosa* often require repeated courses of intravenous antibiotics, many of which may be nephrotoxic. In keeping with this, I have already demonstrated that a significant proportion of adult CF patients have renal impairment due to cumulative life time exposure to such antibiotics (Chapter 4). Although there have been recent reports of episodes of acute renal failure in children with CF (Kovesi et al, 1998; Drew et al, 2002; Drew et al, 2003), there are no reported cases in the adult CF population. I now present 8 cases of acute renal failure (ARF) in patients attending the Liverpool Adult CF Unit (clinic population at the time of completing this study was 185), all of which occurred in relation to the use of intravenous aminoglycosides for pulmonary exacerbations caused by chronic infection with the Liverpool epidemic *P. aeruginosa* strain (LES), which is frequently multiresistant. Other potential contributory factors are discussed. Demographic data is given in table 5.11.1.

The CF unit did not adopt once daily aminoglycoside dosing at the time. Local protocols required measurement of aminoglycoside levels pre and one hour post the fourth administration and dose adjustment to achieve a trough level of < 2.0 mg/l and a peak level of 6-10 mg/l. Subsequently, trough and peak levels were re-measured 24 hours after any dose adjustment, weekly thereafter and as needed in response to changes in the patient's condition to ensure therapeutic serum concentrations. Consistent with general experience (Tan et al, 2002a), adherence to this monitoring protocol was less than ideal during home-based IV antibiotic therapy.

5.2 CASE 1

Case 1 presented in April 2000 with a chest exacerbation with associated musculoskeletal chest wall pain requiring diclofenac. Admission serum urea,

creatinine and electrolytes (U&E) were normal and he was commenced on his usual dose of IV tobramycin 180 mg tds (9.4 mg/kg/day) and IV colomycin 2 megaunits tds. Tobramycin levels estimated pre and one hour post the fourth dose were within the protocol range (<1 mg/l and 7.8 mg/l respectively). His chest improved but after 16 days, routine tobramycin assay revealed trough and peak levels of 18 and 30 mg/l respectively. Urea was raised at 16.8 mmol/l and creatinine at 231 µmol/l. Antibiotics and diclofenac were stopped and IV hydration commenced. There was no evidence of urinary infection and renal ultrasound was normal. Renal function continued to deteriorate until day 20 (peak creatinine 331 µmol/l). U&E slowly returned to normal over the next three weeks but CCl six months later remained reduced at 58 ml/min (lower limit of normal range ≥ 80).

5.3 CASE 2

Case 2 presented in June 2003 with a pulmonary exacerbation associated with pleuritic chest pain. U&E were normal. Spirometric measurement was impossible because of a longstanding Bells Palsy. He was given IV tobramycin 160 mg tds (9.8 mg/kg/day, his usual dose), IV co-trimoxazole (1.44 g bid) (for co-existing longstanding *Burkholderia cenocepacia* infection), IV colomycin (2 megaunits tds) and diclofenac for his pain. Trough and peak tobramycin levels around the fourth dose were satisfactory. On day 10 he complained of dizziness, ataxia and tinnitus. Aural examination was normal. Trough tobramycin level was 8.9 mg/l and renal function was impaired (urea 23.4 mmol/l, creatinine 300 µmol/l). Urine was sterile. Renal USS showed normal size kidneys. Tobramycin and diclofenac were discontinued and IV rehydration commenced with good effect. Serum U&E returned to normal by 21 days, but CCl four months later was only 63 ml/min. CCl had previously been measured in July 2002 when he was clinically stable at 86 mls/min.

5.4 CASE 3

Case 3 was maintained on azithromycin as an anti-inflammatory agent. In November 2003 she started home IV antibiotics (tobramycin 120 mg tds [8 mg/kg/day] and colomycin 2 megaunits tds) for a pulmonary exacerbation. Baseline U&E were normal. Serum tobramycin levels pre and one hour post the fourth dose were appropriate (0.4 and 9.3 mg/l respectively). On day 12 she developed a flu-like illness and widespread inflammatory arthropathy (mainly wrists, fingers and knees). Simple analgesia was ineffective and Ibuprofen was commenced. On day 15 she developed vomiting, dyspepsia, reduced oral intake and a 3 kg weight loss was noted. A random tobramycin level was 14.8 mg/l and urea and creatinine were elevated (16 mmol/l and 265 µmol/l respectively). MSU was negative and renal USS showed normal kidneys with no obstruction.

Ibuprofen, azithromycin and antibiotics were discontinued and IV rehydration commenced. Serum creatinine peaked at 289 µmol/l on day 17 before gradually returning to normal on day 25 (107 µmol/l), at which point CCl was 37 mls/min.

5.5 CASE 4

Case 4 was listed for pulmonary transplantation, and required prolonged courses of IV antibiotics, often carried out at home, to achieve a clinical effect. In October 2000 U&E were normal. In December 2000 she undertook a course of home IV tobramycin (160 mg tds: 10.3 mg/kg/day – her usual dose) and IV ceftazidime (3 g tds) and serum tobramycin levels around the fourth dose were normal (0.9 and 8.3 mg/l respectively). By day 18 there had been little clinical change and sputum cultures suggested reduced susceptibility of her *P. aeruginosa* isolate to ceftazidime, which was accordingly changed to IV colomycin 2 megaunits tds. At day 25, azithromycin was added in and the intravenous antibiotics continued

unchanged. Tobramycin levels remained in the therapeutic range. By day 39, FEV1 had improved but she complained of nausea, headache, dizziness and widespread arthralgia; BP rose to 180/95, renal function deteriorated (urea 42.1 mmol/l and creatinine 394 µmol/l) and a random tobramycin level was high at 39 mg/l. She was admitted to hospital, IV antibiotics and azithromycin stopped, and rehydration started. Investigation of the renal tract was unhelpful except for microscopic haematuria. Marked peripheral blood eosinophilia (16%) was noted. Serum creatinine peaked at 465 µmol/l on day 46, when the CCl was only 11 ml/min. By day 59 she was normotensive and the U&E had returned to normal, but two months later CCl was only 51 ml/min and was still reduced at 58 mls/min two years later.

5.6 CASE 5

Case 5 was a late diagnosis at age 51 years having been treated for asthma and bronchiectasis in a general chest clinic. Awaiting transplant assessment, she was commenced on nebulised tobramycin (TOBI®) and azithromycin.

In February 2004, she was hospitalised for a respiratory exacerbation. TOBI® was replaced with IV tobramycin (120 mg tds: 9 mg/kg/day) and IV colomycin 2 megaunits tds. Pre treatment renal function was normal. Although trough and peak tobramycin levels on days 2 and 4 were satisfactory (1.1 and 9.1; 1.3 and 8.4 mg/l respectively), by day 9 they were elevated (12.3 and 29.1 mg/l) and renal function was abnormal (Cr 285 µmol/l). Renal tract investigations were normal, except for urine and peripheral blood eosinophilia. Antibiotics and azithromycin were stopped and she was treated conservatively with IV hydration. Three days later random serum tobramycin was 1.1 mg/l. Recovery of renal function was slow and 24 days later CCl was 41 ml/min. Due to her severe respiratory morbidity nebulised TOBI and azithromycin were restarted four weeks later without immediate adverse effects on renal function.

5.7 CASE 6

Case 6 was admitted in May 2001 for an exacerbation and treated for two weeks with meropenem, colomycin (2 megaunits tds) and tobramycin (120 mg tds: 6.5 mg/kg/day; her usual dose is 140 mg tds). Tobramycin levels were 2.3 and 9.0 mg/l pre and post the fourth dose respectively. Tobramycin dose was not adjusted, and U&E remained normal. In June 2001 she had a further exacerbation, treated at home with tobramycin (120 mg tds), colomycin (2 megaunits tds) and ceftazidime (3 g tds). On day 4 she became pyrexial and developed a widespread macular rash. Trough and peak tobramycin levels were 6.0 and 18 mg/l respectively. Urea was 10.5 mmol/l and creatinine 318 µmol/l. There was no evidence of a urinary tract infection and renal USS was unremarkable. A blood eosinophilia of 11% and haematuria and eosinophilia in the urine sediment suggested a drug-induced allergic reaction. Antibiotics were stopped and she insisted on discharge. U&E returned to normal after two weeks, although on day 19 CCl was only 60 ml/min.

5.8 CASE 7

Case 7 was taking azithromycin as an anti-inflammatory agent. In June 2000 she was treated at home with gentamicin (120 mg tds: 6.6 mg/kg/day) and meropenem (1 g tds). Gentamicin levels were within the therapeutic range (trough 1.0 and peak 8.3 mg/l) and U&E were normal. She did not improve and on day 10 was hospitalised. At day 15 gentamicin levels were 6.0 and 9.4 mg/l pre and post dose respectively, on unchanged medication. Creatinine rose to 144 µmol/l. Gentamicin was stopped and subsequently restarted (she would not accept an alternative in view of previous allergic reactions or intolerance to many antibiotics) at 120 mg bid with satisfactory levels thereafter. She was discharged with clinical improvement and normal U&E at day 31. She represented two weeks later with a further exacerbation. On this occasion she was treated with tobramycin (140 mg tds: 7.6 mg/kg/day) and meropenem (1 mg tds): pre and post fourth dose tobramycin levels were 7.9 and 16.8 mg/l respectively and tobramycin was omitted for several doses and then

recommenced at 80 mg bid on day 7. Subsequently tobramycin levels remained in the therapeutic range (pre dose <1 and post dose 8.7-10.1 mg/l) for multiple assays taken between days 8 and 22. The first abnormal creatinine was noted on day 8 (157 µmol/l) and reverted to normal by day 14. Investigation of the renal tract failed to provide an alternative explanation. Furthermore, on day 22 she complained of dizziness, tinnitus, and deafness, and ENT review revealed disequilibrium and high tone sensorineural deafness in keeping with aminoglycoside toxicity. Tobramycin treatment was withdrawn.

5.9 CASE 8

Case 8 underwent ureteric reimplantation for pelvi-ureteric juction obstruction/strictures and had recurrent urinary tract infections as a young child, but had no episodes of renal dysfunction as an adult. In October 2000 she received two weeks of IV tobramycin (160 mg tds) and colomycin (2 megaunits tds) at home for a pulmonary exacerbation with good effect and no adverse reactions. The same treatment was repeated five weeks later for a second exacerbation. Peak tobramycin levels > 10 mg/l on days 2 and 5 resulted in the dose being reduced in a stepwise fashion to 100 mg tds. A trough tobramycin assay of 2.9 mg/l on day 10 led to further reduction in dose to 100 mg bid. U&E remained normal throughout. By day 14 her respiratory symptoms and FEV1 had improved but she complained of lethargy, nausea, vomiting, fever, rigors and cloudy urine. She was immediately admitted to hospital and the first abnormal U&E recorded (urea 12.2 mmol/l; creatinine 190 µmol/l). Despite abundant pus cells in the urinary sediment, MSU and blood cultures were sterile. A small calculus was projected over the lower pole of the left kidney on a plain abdominal x ray. USS showed both kidneys to be of normal size without significant scarring or obstruction but the appearances of the parenchyma were in keeping with bilateral pyelonephritis. Immunology screen was negative. She was rehydrated with IV fluids and tobramycin was replaced with meropenem. Her symptoms settled and U&E returned to normal over the next 7

days, although CCl was reduced to 12 ml/min. Renal impairment was still evident 27 months later (CCl 48 ml/min).

5.10 DISCUSSION

The potential for aminoglycosides to cause renal damage was first described in 1945 (Hinshaw and Feldman, 1945). In common with other renally excreted drugs, their serum concentrations are reduced in patients with CF (Rey et al, 1998), who subsequently require high doses to achieve therapeutic levels often over prolonged courses. CF patients are therefore at particular risk of aminoglycoside toxicity. Surprisingly however, there are few reports of renal disease in CF, and acute renal failure has only recently been described in children in association with gentamicin (Drew et al, 2002; Drew et al, 2003). This report is the first to describe aminoglycoside toxicity in adult CF patients, mainly in association with tobramycin which is, traditionally, thought to be one of the less nephrotoxic aminoglycosides (Burkle, 1986; Pedersen et al, 1987).

Aminoglycosides have a narrow therapeutic range, and require careful monitoring of serum levels to prevent acute toxic effects. Such toxicity results from a proportion of the administered dose accumulating in the epithelial cells of the proximal renal tubules, causing a stepwise alteration of cell function ultimately leading to acute tubular necrosis (Mingeot-Leclercq and Tulkens, 1999). Advancing toxicity is characterised by increased electrolyte leak and finally renal failure is manifest by uraemia and high serum creatinine concentration. In clinical practice, oliguric or anuric renal failure is rare. Tubular damage may continue for several days after cessation of aminoglycoside therapy due to high parenchymal levels (Mingeot-Leclercq and Tulkens, 1999). Indeed serum creatinine continued to rise for a few days after aminoglycoside withdrawal in cases 1, 3 and 4 in this series, and in some recovery was protracted. None of the patients presented here had oliguria/anuria, abnormal serum electrolyte concentration or evidence of

glomerulonephritis, and renal biopsy was not performed since all cases recovered without the need for dialysis. However, all were left with reduced creatinine clearance (mean 52 mls/min [range 37-63]; mean interval from episode of ARF 9 months [range 1-27]). In every case, serum urea and creatinine levels recovered to normal after the acute event, highlighting the insensitivity of these routine serum markers for monitoring long term change in renal function.

Aminoglycosides are powerful antipseudomonal agents, and have an important role in the treatment of patients with CF, especially where multiresistant strains are present. In this series, all patients were chronically infected by such a multidrug resistant transmissible strain (the Liverpool epidemic strain) that is generally only sensitive to tobramycin and colomycin (Mirakhur et al, 2003). However, the reasons why these patients developed acute renal toxicity related to aminoglycoside use after many previous courses are unclear. Risk factors for aminoglycoside toxicity include cumulative exposure, high dosage, prolonged therapy, dehydration, concurrent nephrotoxic medication and pre-existing renal disease. All patients presented here had previously received many courses of IV aminoglycosides without overt ill effect (mean 6.8 courses in the preceding 2 years [range 4-11]), and their dosage had been correctly monitored (average 10 satisfactory paired serum tobramycin levels per patient [range 6–18]). Their pulmonary function and nutritional state did not change significantly over this period. Also, the dosages of aminoglycoside were within the UK CF Trust guidelines (Cystic Fibrosis Trust Antibiotic Group 2002) (mean 8.8 mg/kg/day, [range 6.5-10.3]), and with the exception of case 4 (duration 39 days), none were subjected to unusually prolonged treatment (mean 9.8 days [range 4-16] at diagnosis of acute renal toxicity). The close proximity of 2 courses in cases 6, 7 and 8 may have played a part in precipitating renal injury. Only one patient had evidence of pre-existing renal anomaly and none had features of obstructive uropathy or connective tissue disorder/vasculitis.

There are several possible contributory factors potentiating the development of acute renal failure in this series of patients. All patients were on combination antibiotic therapy in keeping with current best practice for CF (Cystic Fibrosis Trust Antibiotic Group 2002), and β-lactam antibiotics have been implicated in drug induced acute tubulo-interstitial nephritis (TIN) (Pusey et al, 1983). In theory, acute TIN may complicate CF as a result of an allergic reaction to many drugs (Stephens and Rigden, 2002), but there is only one report of this complication in CF patients, in relation to the use of ceftazidime (Drew et al, 2002). In my study cohort, review by a senior renal specialist concluded that the substitution of ceftazidime for meropenem (case 6) and the introduction of azithromycin (cases 4 and 5) may be relevant: these cases developed peripheral blood eosinophilia and renal sediments in keeping with drug induced TIN (Pusey et al, 1983). Azithromycin, a macrolide antibiotic increasingly used for its immune modulatory effect in CF, has also been shown to precipitate TIN (Mansoor et al, 1993), but this has not been previously described in CF patients. Also, there are no reports of an interaction between azithromycin and aminoglycosides, and the pharmacokinetics of azithromycin are not significantly altered in patients with mild to moderate renal insufficiency (Hoffler et al, 1995). None of my patients taking azithromycin were on statins, cyclosporin or other immunosuppressive therapy, as interactions with these agents have been reported to induce ARF (Ljutic and Rumboldt, 1995; Grunden and Fisher, 1997). All but two of the cases described above were co-treated with IV colistin. Although no histological evidence of acute interstital inflammation was seen on necropsy of six non-CF intensive care unit patients after treatment with large doses of colistin (26 megaunits per day) (Price and Graham, 1970), suspected acute interstitial nephritis induced by colistin was more recently reported in a 35 year old critically ill non-CF patient upon retreatment with this antibiotic (Kallel et al, 2005) . The authors proposed that he had developed an allergic reaction to colistin after the first exposure manifesting as acute renal failure. However, polymyxin-induced TIN has never been described in the CF literature (Bosso et al, 1991). Obviously, as TIN-induced renal impairment results in increased serum aminoglycoside levels, it

will further exacerbate aminoglycoside related nephrotoxicity. TIN is unpredictable and can happen at any time after introduction of the drug (Pusey et al, 1983).

In addition, many of my patients were also receiving concurrent non-steroidal anti-inflammatory drugs (NSAIDs), which have previously been reported to interact with aminoglycosides in CF patients (Kovesi et al, 1998; Scott et al, 2001). These are frequently administered in adult CF patients, who may present with posture-related musculoskeletal chest or back pain as well as CF-related arthropathy. NSAIDs inhibit intra-renal production of the vasodilator prostaglandin E2 and prostacyclin, causing renal vasoconstriction and acute renal dysfunction (Whelton, 1995). This is relevant only in extracellular volume contracted states, when renal blood flow is dependent on prostaglandin production. It is therefore essential to monitor renal function in CF patients taking NSAIDS particularly if they are unwell, dehydrated and receiving IV aminoglycosides (cases 1, 2 and 3).

Thus, aminoglycoside-induced nephrotoxicity may not be a simple process and can be influenced by several interrelated factors. Indeed, the development of ARF in this case series is very likely multifactorial, with dehydration and the co-administration of several potentially nephrotoxic medicines, such as anti-inflammatory agents and antibiotics, making significant contributions.

The cases discussed above illustrate one of the difficulties encountered in treating adult CF patients infected with multiresistant epidemic *P. aeruginosa*, in particular the LES. This strain is highly prevalent amongst CF patients in Liverpool, and is widespread throughout CF clinics in the UK (Scott and Pitt, 2003). Prevention of the spread of such organisms within the CF community is essential, and this can only be achieved through the implementation of strict segregation policies. Avoidance of aminoglycosides is rarely an option as the emergence of these resistant strains

compels their use. Instead, I suggest that emphasis should be on reducing their harm. For example, compared with the multiple daily dosing schedules of aminoglycosides that were used in all the cases described in this study, once daily regimens were recently shown to be equally effective in the treatment of CF pulmonary exacerbations, with the possible added advantage of reduced nephrotoxicity (Smyth et al, 2005). However, the renal sparing effects of this approach were only seen in children but not adult CF patients. In fact, CF adults in this trial displayed a significantly higher serum creatinine after once daily therapy but the study did not have sufficient power to exclude greater nephrotoxicity in the adult subgroup (Smyth et al, 2005). The same authors have since demonstrated reduced tobramycin elimination rate on once daily therapy: this may be explained by early renal damage caused by the higher tobramycin boluses that could not be detected by the crude biochemical assays used in the initial randomised trial (Touw et al, 2007).

Renoprotective strategies could also include choosing less toxic aminoglycosides (Tan et al, 2002a), alternating classes of antipseudomonal antibiotics to increase the interval between course exposures to allow complete drug clearance, and increased vigilance when aminoglycosides are used concurrently with other potentially nephrotoxic drugs. Careful monitoring of drug levels is paramount, particularly if there is a potentially dehydrating state or pre-existing renal anomaly. I have previously demonstrated that adult CF patients have occult renal damage associated with long-term exposure to aminoglycosides (chapter 4). In those paediatric CF units where routine three monthly IV antibiotic courses are offered (which has not been shown to be beneficial (Elborn et al, 2000)) aminoglycosides should be avoided where possible. As survival in CF continues to improve, the incidence of drug-induced renal injury may increase, strengthening the case for screening/surveillance for aminoglycoside-induced toxicity (Tan et al, 2002b). Measurement of creatinine clearance and an assessment of the interval aminoglycoside exposure at each annual screen may be a useful aid.

This work has been published

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5.11 Tables

Table 5.11.1 - Patient Demographics

Multiresistant = resistant to at least 2 of the 3 classes of antipseudomonal antibiotics. This definition excludes colomycin.

Case	Sex	Age	Genotype	FEV1 % predicted	BMI	Diabetes	*Pseudomonas* antibiogram	IV courses per year
1	M	20	DF508/DF508	54	19.1	1 year	Multiresistant	5
2	M	42	DF508/DF508	Bells Palsy	20.2	No	Multiresistant (Also *B cenocepacia*)	6
3	F	21	DF508/DF508	46	19.2	2 years	Multiresistant	3
4	F	20	DF508/DF508	43	20.0	No	Multiresistant	8
5	F	52	DF508/R117H	30	15.5	2 years	Multiresistant	2
6	F	19	DF508/DF508	48	20.1	1 year	Multiresistant	4
7	F	19	DF508/DF508	60	19.8	2 years	Multiresistant	4
8	F	18	DF508/DF508	98	23.0	No	Multiresistant	4

CHAPTER SIX

Comparison of methods of assessment of renal function in CF patients

6.1 INTRODUCTION

Although CFTR protein is expressed in the kidney, renal disease is not a primary complication of the CF state (Morales et al, 2000). Previously, renal stones were a recognised feature of dehydration due to CF (Perez-Brayfield et al, 2002), and more recently cases of acute renal failure have been reported in CF children (Kovesi et al, 1998; Drew et al, 2003). I have now shown that acute renal failure can occur in CF patients undergoing aminoglycoside therapy (Chapter 5), and have demonstrated an association between the cumulative lifetime use of these nephrotoxic antibiotics and diminishing renal function in adult CF individuals (Chapter 4). With improving survival, CF patients will become repeatedly exposed to these renally harmful antibiotics in the treatment of pulmonary exacerbations and careful monitoring of renal function is therefore essential.

Renal function depends upon the glomerular filtration rate, which is most easily reflected in clinical practice by the ability of the kidney to clear the muscle breakdown product creatinine (the creatinine clearance rate) (Cameron and Greger, 1997). Measurement of this requires comparison of the urinary and serum creatinine, which in turn depends upon an accurate assessment of urinary creatinine, best provided by a 24 hour urine collection (Cameron and Greger, 1997). However, the need for timed urine collections can lead to compliance errors in adults, and may not be possible in children.

To circumvent this, formulae (Jelliffe, 1971; Jelliffe, 1973; Cockcroft and Gault, 1976; Lott and Hayton, 1978; Mirahmadi et al, 1983; Salazar and Corcoran, 1988; Cronberg et al, 1992; Agarwal and Nicar, 1994; Levey et al, 1999; Rule et al, 2004) based on the serum creatinine (SCr) and physical characteristics of the subject have been developed to estimate creatinine clearance. They are commonly used in children and also where drug dosages need to be adjusted according to renal function. Of these, the Cockcroft-Gault Formula (CGF) (Cockcroft and Gault, 1976)

and the abbreviated Modification of Diet in Renal Disease formula (aMDRD) (Rule et al, 2004) are the most widely used in clinical practice. The accuracy of CGF in different settings (eg, the elderly (Nicoll et al, 1991), diabetics (Sampson and Drury, 1992) and patients with cancer (Davila and Gardner, 1987)) has been documented. CGF has recently been recommended for use in CF patients (Tan et al, 2003) and the UK Chronic Kidney Disease guidelines (Burden and Tomson, 2005) advocate the use of aMDRD for automated laboratory reporting of estimated glomerular filtration rate. However, neither of the two formulae has been validated in the CF population. To investigate this further, I compared the accuracy of CGF, MDRD, aMDRD and other formulae with measured creatinine clearance over a range of renal function in a group of adult CF patients and also in a control group of non-CF subjects.

6.2 PATIENTS AND METHODS

6.2.1 CF population

Eighty three adult CF patients (FEV1 % predicted: mean 63, standard deviation [SD] 23, range 16-119) attending the outpatient clinic were recruited over a 26 month period. All patients had a serum creatinine within the normal range (mean 85.5 µmol/l, SD 15.8; local laboratory reference range is 50-140 µmol/l). None had previous history of renal problems, including acute drug-related nephrotoxicity, and all were in a stable clinical state with no acute antibiotic therapy for at least 12 weeks prior to study. In addition, all had normal renal ultrasound scans at annual screen.

6.2.2 Control population

The control group consisted of forty age and BMI matched (table 6.5.1) non CF subjects attending the general medical department for management of hypertension with stable renal function and a serum creatinine within the normal range (84.0 µmol/l [SD 13.5]). None had severe hypertension, any evidence of end

organ damage, or were taking nephrotoxic drugs. Controls were selected if they had at least one urine sample for estimation of GFR collected during the same time interval as that for the CF group.

6.2.3 Measured creatinine clearance (mCCl)

All subjects received routine instructions on how to collect 24 hour urine samples. CF patients submitted 206 urine collections (median 2 samples per patient; range 1 – 5) compared with 82 collections from controls (median 2 per patient, range 1-3). Samples of less than 500 ml were excluded. The mean urinary creatinine per kilogram body weight was calculated separately for men (CF: 0.187 [SD 0.046] mmol/kg; controls: 0.181 [0.061] mmol/kg) and women (CF: 0.138 [0.040] mmol/kg; controls: 0.142 [0.044] mmol/kg). As previously described (Thakur et al, 1997; Chapter 4), all 24-hour urine collections that had creatinine/kilogram within one standard deviation of these respective means were considered valid and included for further evaluation. Following validation, 159 (77%) collections from 74 CF patients and 60 (73%) from 29 controls were used to obtain mCCl. Valid collections from each patient were averaged to form single data points. SCr was measured after an 8 hour fast at the end of each collection by standard autoanalyser technique. The clearance of endogenous creatinine was determined by the formula mCCl = Urine Cr * Urine volume / SCr and expressed as ml/min (Cameron and Greger, 1997). No attempt was made to convert mCCl in terms of body surface area (BSA).

6.2.4 Estimated creatinine clearance (eCCl)

The age, sex and weight of each subject were determined. eCCls were calculated according to the formulae as listed in appendix I (Jelliffe, 1971; Jelliffe, 1973; Cockcroft and Gault, 1976; Mirahmadi et al, 1983; Salazar and Corcoran, 1988; Cronberg et al, 1992; Agarwal and Nicar, 1994; Levey et al, 1999; Rule et al, 2004).

Gender correction factors were applied when indicated. If a formula estimated CCl per 1.73 m^2 BSA, the estimate was readjusted for the subject's actual BSA prior to any analysis. Predictions were made without knowledge of mCCl.

6.2.5 Statistical analysis

Results were expressed as the mean +/- SD. For each formula, mean eCCl and its SD, Pearson's correlation coefficient (r), p value, and bias (mean eCCl - mean mCCl) was determined. Using methods described by Bland and Altman (Bland and Altman, 1986), 95% confidence interval for bias and limits of agreement (LOA = bias +/- 2SD) were computed. In the case of CGF and aMDRD, these values were also calculated after splitting the data by sex and the presence of renal insufficiency (mCCl < 80 ml/min). For the linear regression analysis, mCCl was plotted on the x-axis vs eCCl on the y-axis (figure 6.6.1). Data from non-CF controls were similarly analysed.

To test the variability of the mCCls in subjects who submitted more than one valid urine collection, a repeated measure ANOVA model was devised, and an F test was used to compare the intra-individual variations of CF and non CF subjects.

6.3 RESULTS

Table 6.5.2 lists the formulae in descending order of r value in the CF group. Defining a better predictor as one with a stronger correlation with the mCCl and a narrower range of error (LOA), CGF and either variety of MDRD were not found to be superior to other formulae. Amongst formulae with single digit bias in the CF group, CGF and Mirahmadi had the narrowest LOA: CGF was likely to overestimate mCCl by as much as Mirahmadi was likely to underestimate it. As CGF and aMDRD are the most commonly used in clinical practice, they were chosen for the further analyses described below.

When CGF eCCl was compared with mCCl in CF subjects, the overall correlation was significant [r = 0.69, p<0.001 (figure 6.6.1)] but less solid than that in non-CF controls [r = 0.85, p<0.001]. Similar values were found for aMDRD (0.56 and 0.88 respectively, p<0.001). Both formulae generated greater mean bias (9.7 and 4.9 vs 3.4 and 1.4 ml/min respectively, p<0.05) and wider LOAs (-25 to 44 and -35.3 to 45.1 vs -24 to 31 and -23.6 to 26.4 ml/min respectively) in CF patients compared with non-CF controls. These are shown as Bland and Altman plots (figure 6.6.2). Furthermore, the proportion of CGF- and aMDRD-derived eCCl values within 10%, 20%, and 33% of mCCl for CF patients were only 25, 54 and 78% and 27, 56 and77% respectively compared with 35, 72 and 95% and 62, 85 and 97% for controls (CGF: p<0.05 and <0.01; aMDRD <0.001 and <0.01 at the 20% and 33% thresholds respectively, figure 6.6.3). MDRD produced similar results to aMDRD (data not presented).

Thirty five CF patients had diminished mCCl compared with 8 non CF controls (47% vs 28%, p<0.01), and significantly more mCCl readings were <80 ml/min in the CF group (72 out of 159 (45%) vs 13 out of 60 (22%), p<0.01). In CF patients, both CGF and aMDRD grossly overestimated CCl in the presence of renal insufficiency (table 6.5.3): the bias was very large (18 and 16 ml/min respectively) and the limits of agreement were wide (-10 to 46 and -18 to 50 respectively), considering that the mean mCCl in this subgroup was only 63 ml/min. Conversely, when mCCl was normal (≥ 80 ml/min), CGF and aMDRD were much more accurate predictors of CCl (mean bias 1.6 and -5.6 ml/min respectively, p<0.001). In contrast, both formulae produced very similar results for both men and women (table 6.5.4). For the non CF group, figures were similar to overall CF data summarised in tables 6.5.3 and 6.5.4.

In both the CF and the control groups the first and subsequent mCCl were not significantly different from one another and the intra-individual variation of repeat mCCl was similar in both groups (P=NS for both groups).

6.4 DISCUSSION

With increasing reports of acute drug-related renal injury in CF patients, surveillance for aminoglycoside nephrotoxicity is advocated (Wood et al, 1996; Tan et al, 2003; Smyth et al, 2008). How best to monitor renal function in this patient group is unclear. Serum parameters including creatinine and electrolyte levels, such as magnesium, are both insensitive and unreliable (Shemesh et al, 1985; Levey et al, 1988; Akbar et al, 1999). They may remain within the normal range despite significant and otherwise undetected loss of glomerular filtration and/or tubular function. Indeed in this study, all CF patients retained normal serum creatinine concentrations despite 35 having subnormal mCCl.

Accurate measurement of glomerular filtration relies upon a chemical that is merely filtered through the glomerulus, without any active tubular reabsorption or secretion. Inulin is such a compound, and its clearance is considered the "gold standard" for measuring the glomerular filtration rate (Cameron and Greger, 1997). However, its use in day to day clinical medicine is impractical. The use of the endogenous substance creatinine (which has a constant and steady production from muscle breakdown during periods of stable body weight and dietary protein intake) is an acceptable alternative, although some active secretion in the tubule may falsely elevate the creatinine clearance rate above GFR by a factor of 1.1 (Bauer et al, 1982a). This effect is exaggerated as GFR falls and the serum creatinine rises (Bauer et al, 1982b; Shemesh et al, 1985; Giovannetti and Barsotti, 1991). Nevertheless, in clinical practice measured creatinine clearance is used in lieu of inulin clearance to measure GFR. I therefore used this measurement as the standard against which formulae-derived estimates were judged. Although using it I showed that up to 40% of the CF group had impaired renal function, the true number with a diminished GFR is likely to be greater. I was therefore assured that using this method did not overestimate renal problems in my patients.

One possible criticism of this study is the use of timed urine collection to derive creatinine clearance, where failure to collect all urine in 24 hours can result in an underestimate of GFR. To avoid this, only specimens of adequate volume (> 500mls) were included (Cockcroft and Gault, 1976). I could not find a published reference range for normal daily creatinine excretion in adult CF patients: to ensure that outlying creatinine values did not bias the results, I only included samples within one standard deviation of the mean value, of all pooled samples per gender, for total 24 hour creatinine excretion corrected for body weight, a method used by other workers (Thakur et al, 1997). Additionally, thus labelled "valid" specimens generated reproducible results on repeat measurement. I am therefore confident that the technique employed in this study for timed urine collection allowed an accurate measurement of creatinine clearance, in turn reflecting the GFR.

Nevertheless, this method of measuring creatinine clearance in clinical practice is cumbersome; in adults poor compliance can lead to collection errors and in children collection may be difficult. In an attempt to overcome this, several formulae (Jelliffe, 1971; Jelliffe, 1973; Cockcroft and Gault, 1976; Lott and Hayton, 1978; Mirahmadi et al, 1983; Salazar and Corcoran, 1988; Cronberg et al, 1992; Agarwal and Nicar, 1994; Levey et al, 1999; Rule et al, 2004) have been developed that claim to approximate creatinine clearance from serum creatinine concentrations by factoring in the patient's biometric data with a range of constants. Recent guidelines (K-DOQI (National Kidney Foundation, 2002) and UKCKDG (Burden and Tomson, 2005)) suggest that such formulae should be used to estimate GFR in preference to mCCl. All these formulae are predictors of the measured CCl, and typically generate a range wherein mCCl falls. The more accurate the formula, the smaller this range: an ideal predictor should have a range equal to zero and a correlation coefficient (r) equal to one. In practice, however, all known formulae are associated with some error, and the aim is to select a predictor that correlates well with mCCl with the minimum of error. Bland and Altman demonstrated that a positive correlation does not always translate into a good prediction, nor does a

high r value alone guarantee an accurate prediction (Bland and Altman, 1986). Instead, they described a more reliable way of comparing 2 methods of measuring a clinical variable (Bland and Altman, 1986). They documented the difference between the predicted value and the measured value and devised limits of agreement (mean difference +/- 2 SD). Investigators can then decide if this interval is acceptable within their clinical setting. Using these definitions, the first 7 formulae in table 6.5.2 could be equally applicable in CF patients, and none is superior to the others.

I paid CGF and aMDRD particular attention since they are the most commonly used formulae to estimate eCCl in clinical practice. In their original monologue Cockcroft and Gault demonstrated an r = 0.83, with 67% and 95% of their predictions falling within 20% and 35% respectively of the mCCl (Cockcroft and Gault, 1976). Other authors have reproduced similar results, supporting the applicability of these formulae in several disease groups (Davila and Gardner, 1987; Rhodes et al, 1987; Nicoll et al, 1991; Sampson and Drury, 1992; Cochran and St John, 1993; Alcantara et al, 1998) and the use of CGF in assessing renal function in CF was recently recommended (Tan et al, 2003). In our series however, CGF-derived eCCls compared less favourably with those for non-CF controls with a lower correlation with mCCl (r = 0.69 *vs* 0.85) and a wider error range (-25 to 44 *vs* -24 to 31). Additionally, significantly fewer CF estimates fell within 20% and 33% of mCCl (54% *vs* 72% and 78% *vs* 95%, respectively). The results for aMDRD were similar.

There are several possible explanations for the apparent discrepancy between CGF and aMDRD accuracy in CF patients compared with that in controls and other disease groups.

Firstly, since serum creatinine is dependent upon muscle mass, individuals with a diminished muscle mass may have lower serum levels, resulting in a falsely high eCCl and greater bias. This was shown in patients after spinal injury, where paraplegia causes limited muscle mass relative to total body weight. The use of CGF in these patients was studied by Mirahmadi et al (Mirahmadi et al, 1983) who found a large bias of 20 ml/min and suggested a correction factor of 0.8 to account for this overestimation. CF patients may similarly have a reduced muscle mass compared with weight matched controls due to their nutritional difficulties (Stettler et al, 2000; Groeneweg et al, 2002), a hyper catabolic state from chronic systemic inflammation (Vinton et al, 1999), and frequently a limited exercise capacity (Selvadurai et al, 2003). Indeed, The formulas of Cockcroft and Gault, Jelliffe I and Jelliffe II all overpredicted creatinine clearance in a previous small study of 18 CF patients with biases of 19, 24 and 8 ml/min and precisions of 37, 42 and 33 ml/min respectively (Town et al, 1996).

Secondly, it has been shown that formulae overestimate creatinine clearance where there is renal impairment. For example, in a study of the use of CGF in paraplegics, 30% of eCCls were outside 33% of mCCls in the subgroup with renal impairment (Thakur et al, 1997), and this has been reproduced by other authors (Guasch et al, 1996). Thus, the significantly higher proportion of urine collections generating subnormal mCCl in my CF patient group than in the control group or in the study of Thakur et al (45% *vs* 22% and 18% respectively, $p<0.01$) may have contributed to the overestimation produced by CGF. With as many as 40% of eCCl falling outside 33% of the corresponding mCCl for CGF (and 35% for aMDRD) my results show that these formulae are less accurate in predicting creatinine clearance in CF patients with compromised renal function, the very group in which this prediction is of greatest importance.

Thirdly, subtle differences in the renal physiology of CF patients are known to exist but are not well understood (Morales et al, 2000): the enhanced drug clearance in the CF kidney (Rey et al, 1998) is one such variation. It may be that one or more (as yet unknown) variables or constants specific to the CF condition need to be factored into such formulae in order to improve the prediction accuracy.

The simplicity of their use makes such formulae attractive tools in daily clinical practice, but they have one major drawback: the formula is not reliable when serum creatinine is unstable (Cockcroft and Gault, 1976), thus overestimating GFR when serum creatinine is rising and vice versa. Hence I ensured that all subjects in this series were clinically well with stable renal function. The lack of significant variability on repeat measurement of creatinine clearance further supports this.

In summary, although CGF and aMDRD are as effective as other formulae in estimating GFR in adult CF patients, their accuracy is limited and is not as robust as the traditional timed urine collection method when carefully supervised. Formulae have found a niche in calculating drug dosage in renally impaired patients (Preston et al, 1995), and recent guidelines have suggested that they are used in preference to timed urine collections. However, in the context of regular surveillance for aminoglycoside nephrotoxicity, I believe they should be applied with caution as they may significantly overestimate creatinine clearance and hence renal function in this subgroup of CF patients; failing to identify the very patients they are aiming to detect in clinical practice. As awareness of drug-related renal insufficiency in CF increases, so does the need for a more accurate predictor of creatinine clearance in these patients and based on my data I cannot recommend current formulae, including aMDRD and that described by Cockcroft and Gault, as a substitue to carefully supervised direct measurement of creatinine clearance at the present time.

This work has been published

Al-Aloul M, Jackson M, Bell G, Ledson M, Walshaw M. Comparison of methods of assessment of renal function in cystic fibrosis (CF) patients. *J Cyst Fibros*. 2007; 6(1):41-7.

6.5 TABLES

Table 6.5.1 - Baseline characteristics of the study population

Data presented as means (SD) where appropriate

	CF patients	Non-CF controls
N	83	40
M:F	44:39	26:14
Age (yrs)	24.3 (7.5)	26.2 (5.1)
BMI (kg/m^2)	21.3 (3.6)	22.5 (4.3)
SCr (μmol/l)	85.5 (15.8)	84 (13.5)

Table 6.5.2 - Formulae derived eCCl (ml/min) and their relationship with mCCl in CF patients (mean mCCl (SD): 83.1 (22.9) ml/min)

Formula	Mean eCCl (SD)	r	P value	Bias	95% CI for Bias	LOA
Agarwal	93.9 (21.9)	0.7	<0.001	10.8	6.8 to 14.8	- 23.6 to 45.2
Cronberg	95.2 (20.2)	0.7	<0.001	12.1	8.1 to 16.1	- 21.7 to 45.9
Mirahmadi	74 (16.1)	0.69	<0.001	- 9.1	- 13 to – 5.2	- 42.3 to 24.1
CGF	92.6 (20.1)	0.69	<0.001	9.7	5.5 to 13.5	- 24.9 to 43.9
Jelliffe 2	87 (20.8)	0.67	<0.001	3.9	- 0.2 to 8	-31.7 to 39.5
Lott	88.1 (19.3)	0.61	<0.001	5	0.6 to 9.4	- 32.4 to 42.4
MDRD (6 variable)	87.1 (19.2)	0.60	<0.001	4	- 0.5 to 8.3	- 34.3 to 42.3
Jelliffe 1	82.4 (14.9)	0.57	<0.001	- 0.7	- 12 to 10.6	-38.1 to 36.7
aMDRD (4 variable)	88 (19.7)	0.56	<0.001	4.9	0.3 to 9.5	- 35.3 to 45.1
Salazar	106 (22.9)	0.55	<0.001	22.9	17.9 to 27.9	- 20.7 to 66.5

Table 6.5.3 -

Comparison of mCCl and eCCl (CGF and abbreviated MDRD [4 variable]) in renally impaired CF patients (mCCl < 80 ml/min)

	n	Mean mCCl (SD)	eCCl formula	Mean eCCl (SD)	% eCCl within 33% of mCCl	Bias	95% CI for Bias	LOA
mCCl<80	35	63.5 (12.3)	CGF	81.7 (18.1)	60%	18.3	13.6 to 23	- 9.7 to 46.3
			aMDRD	79.3 (20.2)	65%	15.8	10.5 to 22.0	- 17.5 to 50.1
mCCl≥80	39	100.7 (13.8)	CGF	102.3 (16.6)	95%	1.6	- 3.5 to 6.7	- 30.4 to 33.6
			aMDRD	95.1 (16.1)	84%	- 5.6	- 11.1 to - 0.1	- 39.6 to 28.4
total	74	83.1 (22.9)	CGF	92.6 (20.1)	78%	9.7	5.5 to 13.5	- 24.9 to 43.9
			aMDRD	88 (19.7)	77%	4.9	0.3 to 9.5	- 35.3 to 45.1

Table 6.5.4 -

Gender based comparison of mCCl and eCCl (CGF and aMDRD) in CF patients

sex	n	Mean mCCl (SD)	eCCl formula	Mean eCCl (SD)	% eCCl within 33% of mCCl	r	Bias	95% CI for Bias	LOA
M	40	93 (21)	CGF	103 (14)	80%	0.58	10	5 to 16	- 16.6 to 38.2
			aMDRD	97.8 (15)	74%	0.40	4.8	-0.4 to 10	- 44 to 53.6
F	34	72 (20)	CGF	80 (19)	76%	0.58	8	2 to 14	- 27.7 to 43.2
			aMDRD	73.9 (17)	79%	0.62	1.9	- 2.1 to 5.8	- 32.7 to 36.5
total	74	83.1 (22.9)	CGF	92.6 (20.1)	78%	0.69	9.7	5.5 to 13.5	- 24.9 to 43.9
			aMDRD	88 (19.7)	77%	0.56	4.9	0.3 to 9.5	- 35.3 to 45.1

6.6 FIGURES

Figure 6.6.1

a) mCCl and eCCl correlation derived from the Cockcroft-Gault formula in CF patients (r=0.69, P < 0.001; mean bias 9.7 ml/min)

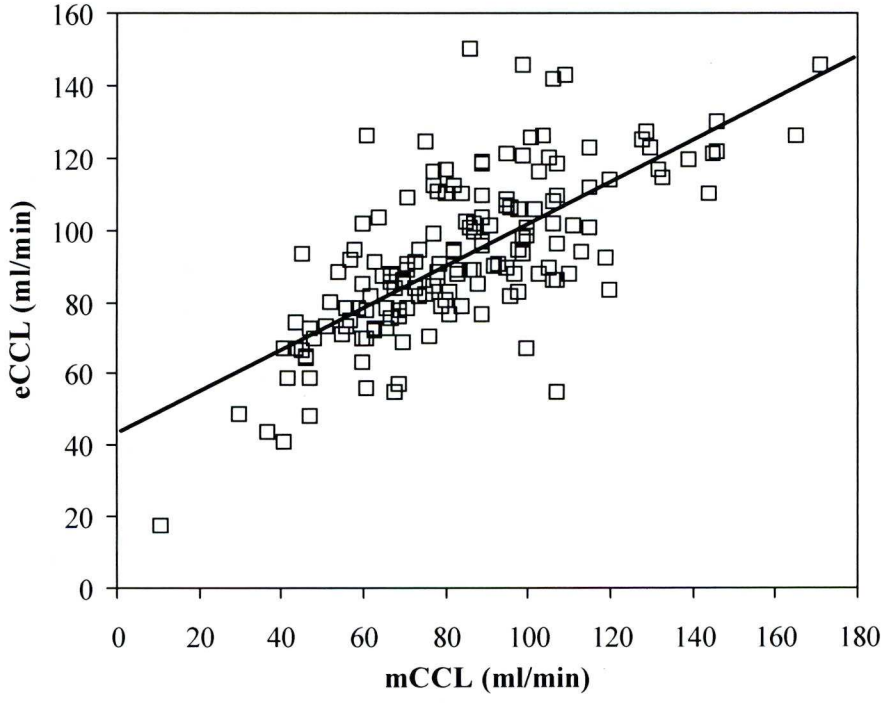


Figure 6.6.1 continued

b) mCCl and eCCl correlation derived from the Cockcroft-Gault formula in non CF controls (r=0.85, P<0.001; mean bias 3.4 ml/min)

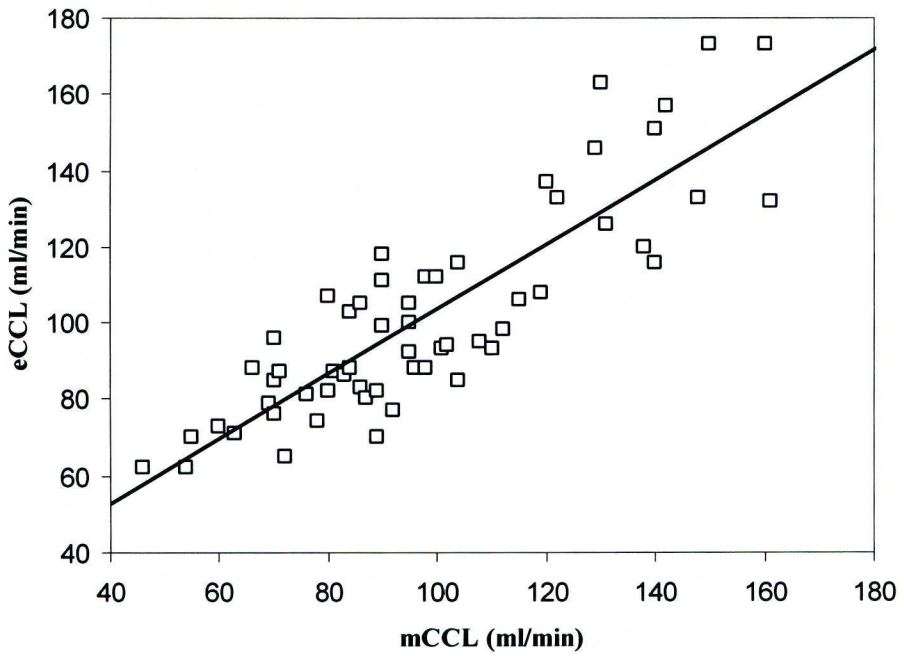


Figure 6.6.1 continued

c) mCCl and eCCl correlation derived from aMDRD in CF patients (r=0.57, p<0.001; mean bias 4.9 ml/min)

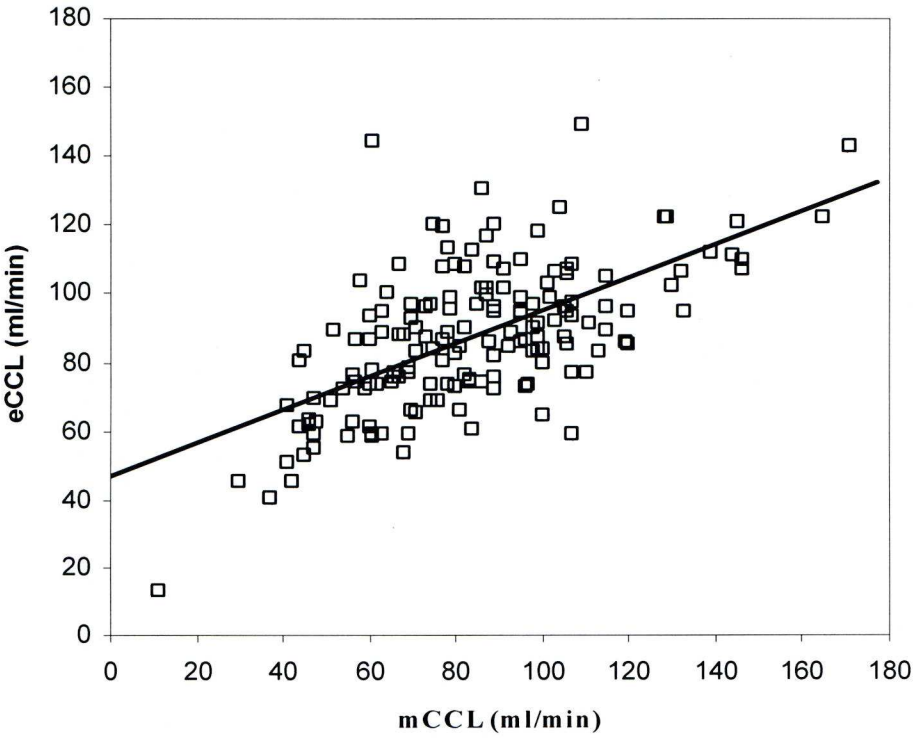


Figure 6.6.1 continued

d) mCCl and eCCl correlation derived from aMDRD in non CF controls (r=0.88, P<0.001; mean bias 1.4 ml/min)

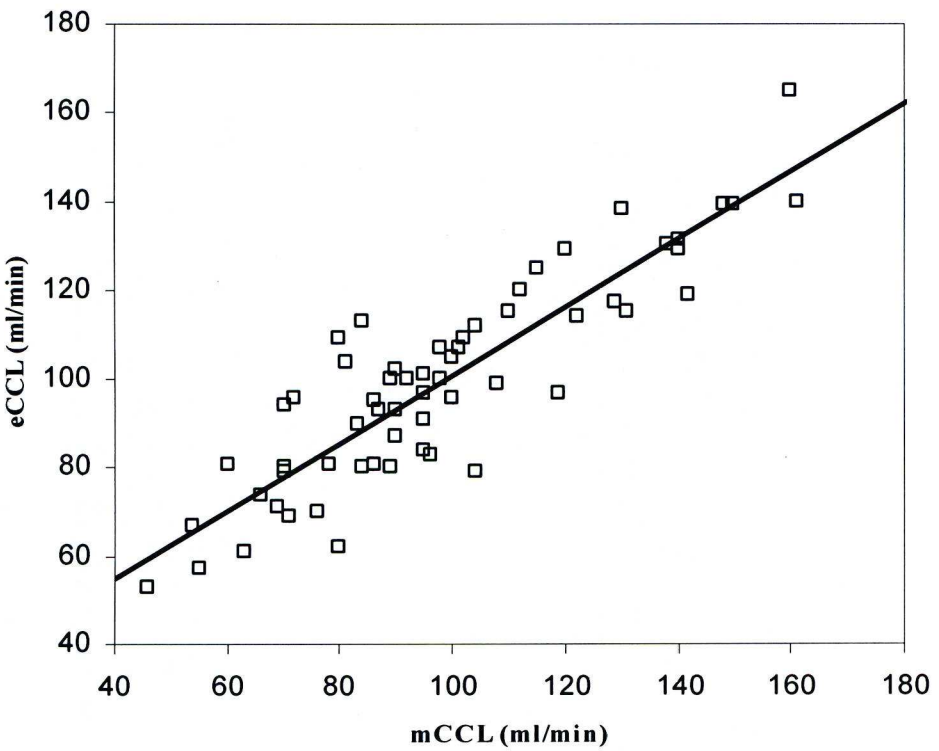
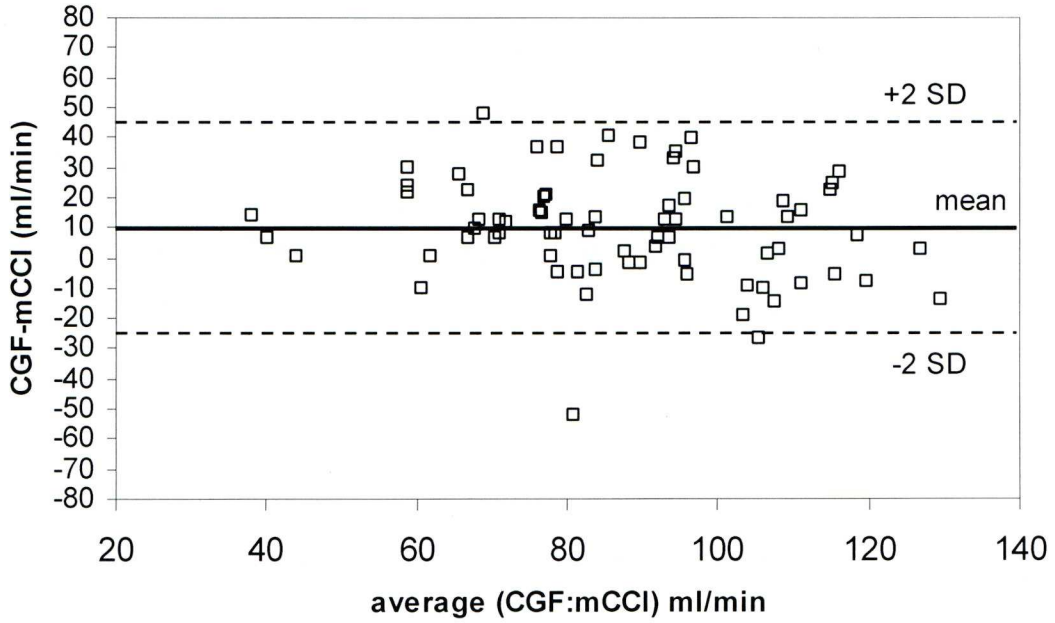


Figure 6.6.2

Bland and Altman Plots comparing mCCl with CGF and aMDRD in CF patients



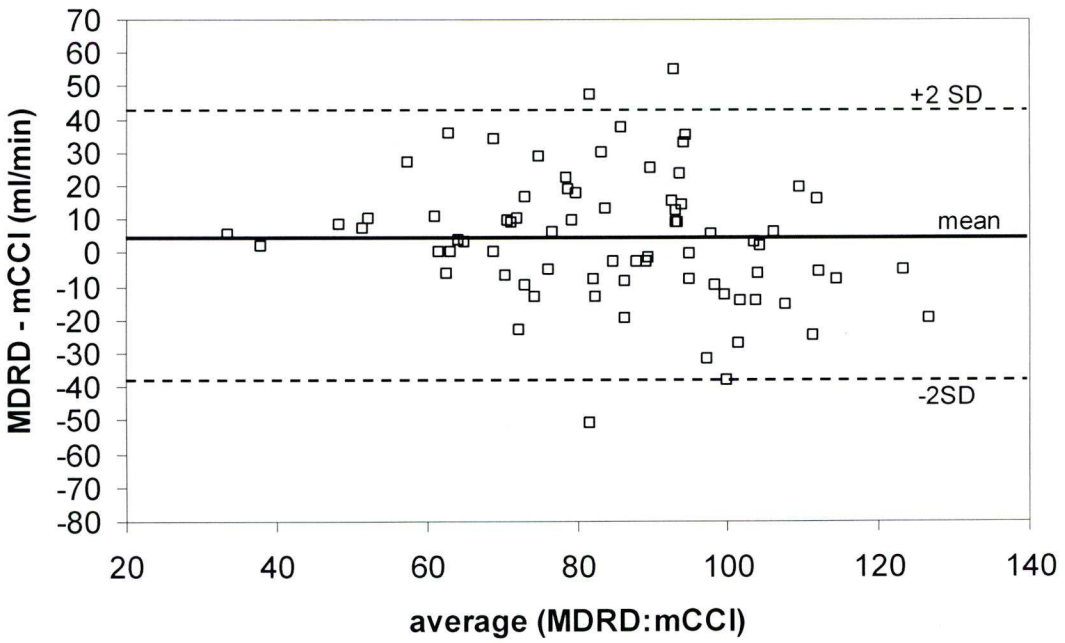
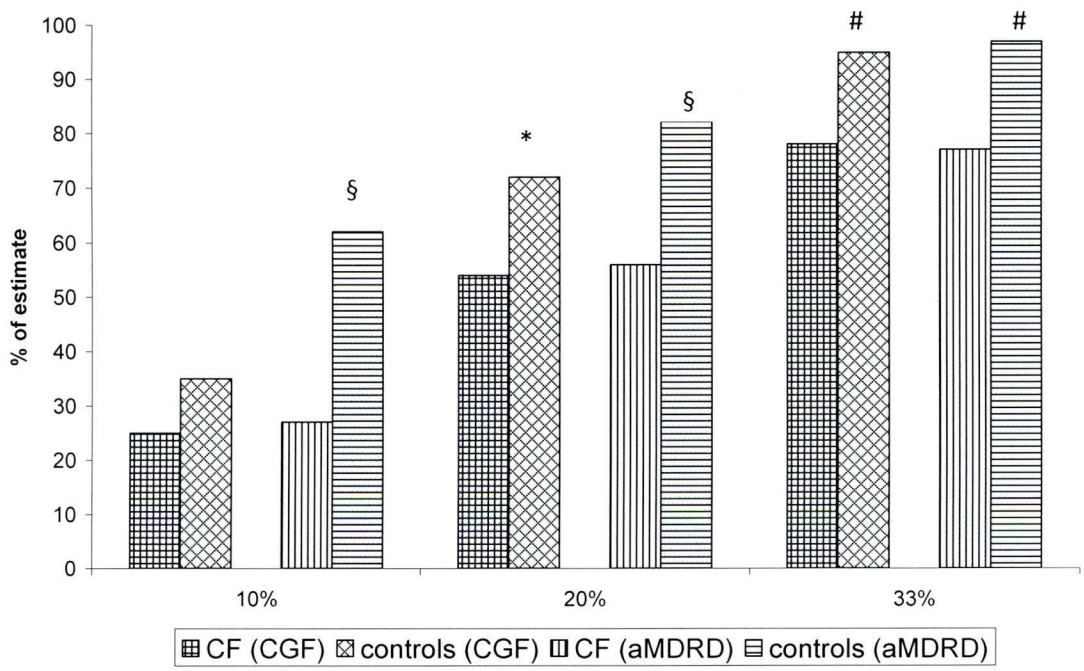


Figure 6.6.3

Percentage of eCCl results falling within 10, 20 and 33% of mCCl in CF patients and non-CF controls. CGF and aMDRD shown.



Chi square analysis (Mantel-Haenzel test)

* p <0.05 *vs* CF

\# p <0.01 *vs* CF

§ p <0.001 *vs* CF

6.7 APPENDIX I.

Formulae for the estimation of CCl reviewed in this study

Author(s)	male	female
CGF 1976	(140-age)*wt / 72*SCr	0.85 * formula for male
Lott 1978	(140-age)*lean wt / 72*SCr	0.85 * formula for male
Jellife 1971	98 - 0.8 (age-20) / SCr	0.9 * formula for male
Jellife 1973	100 / SCr - 12	80 / SCr - 8
Mirahmadi 1983	0.8 (140-age)*wt / 72*SCr	0.85 * formula for male
Agarwal 1994	[28.2 – (0.172*age)]*wt / 14.4*SCr	[21.9 – (0.115*age)]*wt / 14.4*SCr
Cronberg 1992	<70 kg: (170-age)*wt / SCr >70 kg: (160-age)*wt / SCr	(150-age)*wt / SCr
Salazar 1988	[(146-age)*(0.287*wt + 9.74*height^2)] / 60*SCr	[(137-age)*(0.285*wt + 12.1*height^2)] / 51*SCr
MDRD (6 variable)	$186*Scr^{-1.154}*age^{-0.203}$	0.742* formula for male
Abbreviated MDRD (4 variable)	$170*SCr*age^{-0.176}*SUrea^{-0.170}*Alb^{0.318}$	0.762* formula for male

CHAPTER SEVEN

Changes in renal tubular function with repeated exposure to aminoglycosides in CF patients

7.1 INTRODUCTION

In chapter 4, I documented cumulative sub-clinical reductions in creatinine clearance proportional to life time IV aminoglycoside exposure in a cohort of adult CF patients attending the Liverpool CF unit. The Leeds CF group also demonstrated a significant association between higher baseline urinary N-acetyl-β-D glucosaminidase (NAG) and the previous 6-year exposure to tobramycin and colistin (Etherington et al, 2007).

Tobramycin is primarily a proximal tubular cell toxin. Mechanisms for its toxicity include binding to cell membrane phospholipids denuding the apical brush border, sequestration in proximal tubular cell lysosomes and injury to mitochondrial respiration (Kaloyanides, 1992). Most commonly tobramycin produces relatively minor proximal tubular dysfunction leading to release from tubular cells and therefore elevated urinary levels of NAG, a proximal tubular lysosomal enzyme and alanine aminopeptidase (AAP), a brush border enzyme (Mondorf, 1982; Price, 1982). Both NAG and AAP have been widely used as markers of subtle renal tubular damage (Kunin et al, 1978; Diener et al, 1981; Gibey et al, 1981; Davey et al, 1983; Bosomworth et al, 1999). After repeated intravenous courses more marked tubular dysfunction leading to hypomagnesaemia (Akbar et al, 1999), aminoaciduria and glycosuria with loss of urine concentrating capacity can be seen and rarely even acute, non-oliguric, renal failure may ensue (Prime and Tune, 1981).

To explore possible underlying mechanisms for cumulative aminoglycoside toxicity, in the study presented in this chapter I examined changes in renal function during two consecutive CF exacerbations each treated with a standard course of IV tobramycin using a common UK dosage schedule. Enzymuria and proteinuria were used to detect minor changes in proximal tubular function. U&E and CCl were used to gauge more severe alterations in tubular performance.

7.2 PATIENTS AND METHODS

7.2.1 Study population

I recruited 40 consecutive adult CF patients [mean (SD) age: 22.1 (4.7) years, FEV1% predicted: 53 (19) %, BMI: 21.9 (2.4) kg/m^2, 18 males] presenting with an acute pulmonary exacerbation for which IV antibiotic therapy was judged necessary and who did not receive IV aminoglycosides or colistin for at least 12 weeks prior to recruitment.

All patients harboured multiresistant LES *P. aeruginosa* strains which *in vitro* were only predictably sensitive to tobramycin and colistin. Chronic infection was defined as 3 or more positive sputum cultures within the previous 12 months (Cystic Fibrosis Trust Control of Infection Group 2001), and multi-resistance as resistance to at least 2 of the 3 standard antipseudomonal antibiotic classes (Cystic Fibrosis Trust Antibiotic Group 2002). An exacerbation was defined as worsening respiratory symptoms accompanied by spirometric reduction (Rosenfeld et al, 2001a). Patients were excluded if they had known hypersensitivity to aminoglycosides or colistin, significant haemoptysis or new radiographic changes, a history of *Burkholderia cepacia* complex isolation in the preceding 12 months and received any additional oral antipseudomonal antibiotic or non-steroidal anti-inflammatory therapy in the two weeks prior to recruitment.

Changes to maintenance therapy were not permitted within 14 days of the exacerbation. At recruitment, 29 patients were on maintenance nebulised colistin or tobramycin (TOBI®) at home. These were discontinued during inpatient IV antibiotic therapy and recommenced on discharge. Maintenance therapy did not differ between the two treatment episodes. None had fever or engaged in physical exercise for 48 hours prior to recruitment.

Seven had CF-related diabetes at enrolment and one new case of diabetes was diagnosed in the remainder over the study period. Prior to treatment, urine dipstick was negative in all patients and none had pathological proteinuria. None had previous episodes of acute renal failure, all had normal renal ultrasound scans at the preceding annual review and none had received organ transplantation or were prescribed calcineurin inhibitors.

Patients gave written informed consent and the local ethics committee approved the study protocol.

7.2.2 Methods

For each study patient renal function was compared during two consecutive exacerbations requiring repeated treatment with IV tobramycin based on *in vitro* antibiotic sensitivity patterns of their *P. aeruginosa* isolates.

In the 80 treatment episodes studied, all patients received 14 days IV treatment with tobramycin plus colistin, in keeping with the UK CF Trust Antibiotic Policy recommending that exacerbations be treated with a combination of at least two IV antipseudomonal antibiotics (Cystic Fibrosis Trust Antibiotic Group 2002).

For each treatment episode outcome measures included changes from day 0 to day 14 in U&E, CCl (measured from 24 hour urine collections), urinary levels of two proximal tubular cell enzymes (N-acetyl-β-D glucosaminidase (NAG) and alanine aminopeptidase (AAP)) and urinary β_2 microglobulin (β2M) concentrations.

Urinary assays were carried out weekly thereafter until recovery to pre-treatment level. Due to large intra- and inter-individual variability of these urine enzymes and proteins, the following thresholds were pre-defined:

- Complete recovery: urine assay back to the individual's baseline value *or* within 1 SD of the baseline group mean *and* no greater than double the baseline value for each patient. Measurements outside these boundaries represent incomplete renal tubular recovery.
- NAG results were used to categorise patients into complete *vs* incomplete recovery groups, irrespective of corresponding trends in AAP and β2M.

Laboratory methods for U&E, CCl, NAG, AAP and β2M have been described in chapter 3 (General laboratory methods).

7.2.3 Statistical analysis

Unless otherwise specified, data are presented as means and (standard deviations) for metric variables and proportions for categorical variables. For normally distributed data, within- and between-group comparisons were conducted with paired and unpaired t tests respectively. Urinary marker measurements were not normally distributed and therefore Wilcoxon Signed Rank test was used to examine paired baseline data and estimates of the differences between groups were examined using the Mann Whitney U test. Trends in NAG, AAP and β2M were analysed with Pearson's linear correlation. Distributions of renal marker recovery times after the first and second treatments were compared with Wilcoxon Rank Sum test. All tests were 2 tailed and $P \leq 0.05$ were considered significant. Analysis was conducted with SPSS for windows version 15.0.

7.3 RESULTS

Patients' BMI, baseline U&E and CCl were not different in the two treatment episodes (table 7.5.1, figure 7.6.1). Patients received the same dose of tobramycin during both hospital admissions (mean of 7.9 mg/kg/day, SD 1.1, range 5.9 to 9.3 in 2-3 divided doses). Similar serum peak and trough (8.2 *vs* 7.9 mg/l and 0.8 *vs* 1.1 mg/l respectively) tobramycin levels were achieved during the two treatment episodes. A mean of 2 (range 1-4 per patient) paired serum tobramycin levels were collected and no toxic levels were recorded.

After the first treatment, urinary markers, but not U&E, rose significantly (table 7.5.2, figures 7.6.2, 7.6.3a, 7.6.4 – 7.6.6). The changes in NAG, AAP and β2M correlated well (NAG *vs* AAP: r=0.674, P<0.0001; NAG *vs* β2M: r=0.768, P<0.001; AAP *vs* β2M: r=0.508, P= 0.001, figure 7.6.7). The recovery rate of these markers after the first treatment was variable. In 25 patients recovery was complete by week 7 [median 3 weeks, range 1 to 7; figure 7.6.8]. The remainder were re-exposed to IV aminoglycosides prior to complete tubular recovery.

Thirty four (85%) patients completed weekly follow up after the second treatment. Repeat measurements were analyzed accounting for complete (group 1) or incomplete (group 2) recovery of tubular markers prior to aminoglycoside *re-exposure*. The interval between the two consecutive antibiotic treatments is depicted for each group in table 7.5.4.

In group 1, changes in U&E, the rise in tubular markers and their recovery rate were comparable to the first episode (table 7.5.3, figures 7.6.3b-7.6.6, 7.6.9). In contrast, group 2 patients' serum creatinine rose significantly with the second treatment, but it did not cross the threshold of normality (9.4% rise from baseline, p=0.037, figure 7.6.3b). Similarly, Enzymuria and proteinuria were greater than those observed in the first treatment episode (table 7.5.3, figures 7.6.4-6) and their return to baseline

was slower after the second treatment compared with group 1 (median 6 weeks; range 1-8, P=0.0003; figure 7.6.9).

7.4 DISCUSSION

The findings of this study support the impression that IV aminoglycosides can result in acute renal tubular injury even when serum levels are kept within the accepted safe therapeutic range (Trollfors et al, 1980; Tan et al, 2002b; Tan et al, 2003). My data clearly demonstrate that resolution of even mild, otherwise clinically covert, tubular toxicity after cessation of antibiotic therapy could be protracted. Re-exposure to aminoglycosides prior to complete tubular recovery produces incremental tubular toxicity which may explain the previously reported cumulative loss of renal function with frequent aminoglycoside courses (Chapter 4). The different urinary marker signal generated by the two consecutive episodes cannot be explained by differences in aminoglycoside exposure as similar doses and trough/peak levels were observed.

The rise and decline of NAG after the first treatment episode is similar to that previously reported by Glass *et al* (Glass et al, 2005) and Etherington *et al* (Etherington et al, 2007). Both reports described transient rises in urinary concentrations of NAG after a *single* challenge with tobramycin. Glass *et al* described acute tubular injury in 22 CF children and adolescents treated with 2 weeks of tobramycin 3mg/kg tds as monotherapy. This showed evidence of almost complete recovery after 4 weeks, a time scale not too dissimilar to that recorded after the first antibiotic exposure in my study (3 weeks). Etherington *et al* reported an eight-fold rise in NAG in 35 adults exposed to 14 days of IV tobramycin and one of ceftazidime, meropenem, aztreonam or tazocin, all at doses within the UK guidelines. Urinary NAG levels were back to baseline at a median of 52 (range 8-123) days post treatment.

To assess cumulative tubular damage in more detail it would be necessary to repeat these measurements after further courses of tobramycin. In my cohort of 40 patients, the magnitude of peak NAG levels and speed of its subsequent decay over time depended on baseline NAG level prior to the second exposure, which was in turn a function of time lapsed from the first antibiotic treatment. Re-exposure to IV tobramycin within 5 weeks accentuated acute tubular dysfunction and prolonged its resolution whereas an interval of 14 weeks between treatments prevented this. Concordant with this observation, 6 out of 10 patients who received more than one tobramycin course in the study by Etherington *et al* (Etherington et al, 2007) displayed succesive rises in baseline urinary NAG levels, even with a longer interval between treatments (median of 65 days, range 38-150).

Comparison of urinary enzymuria and proteinuria between those with and without CFRD was not plausible due to the small number of patients with CFRD in this study compared with that of Etherington *et al* (Etherington et al, 2007).

I measured two proximal tubular cell enzymes, one localized to the lysosomes (NAG) and another to the apical brush border (AAP) (Mondorf, 1982; Price, 1982). High urinary yield of these enzymes reflects acute microstructural changes caused by a variety of tubulotoxins (Diener et al, 1981; Price, 1982; Davey et al, 1983; Schiavina et al, 1984; Mueller et al, 1989; dos Santos et al, 1994; Bosomworth et al, 1999). β2M was measured to assess potential functional consequences of these structural aberrations. In normal circumstances β2M is virtually completely reabsorbed in the proximal tubule after its filtration at the glomerulus. In the absence of disease states increasing its production by nucleated cells, increased urinary excretion of β2M points to impairment of tubular absorptive capacity and it is therefore regarded a marker of tubular (as opposed to glomerular) proteinuria (Wibell, 1978; Trollfors et al, 1980; Rybak et al, 1987; Tyner, 1999; Kinai and Hanabusa, 2005). Clinico-pathological studies localized aminoglycoside-induced

renal toxicity to the proximal tubule and in particular disruption of lysosomal membrane integrity (De Broe et al, 1989; Mingeot-Leclercq and Tulkens, 1999). Unsurprisingly therefore NAG is the most frequently studied tubular enzyme with the most consistently reproduced reference range. Indeed, a total of eight previous studies reported antibiotic related changes in urinary NAG in CF although a normal range has not been agreed for this patient group (Reed et al, 1981; Steinkamp et al, 1986; Godson et al, 1988; Balla et al, 1998; Ring et al, 1998; Glass et al, 2005; Etherington et al, 2007; Halacova et al, 2008). Hence, in my cohort, changes in NAG signal were used to define the extent of tubular recovery after the first exposure to IV aminoglycosides as either complete or incomplete, irrespective of concommitent changes in AAP and β2M. Reassuringly however, assays of the three urinary markers correlated very well.

The significance of this transient enzymuria and proteinuria had been previously questioned in the absence of clinically measurable functional impairment (Steinkamp et al, 1986). For example, the insult to the renal tubules after a single course of aminoglycosides was not sufficient to produce hypomagnesaemia, elevated serum creatinine or diminished predicted GFR (Schwarz formula) in previous studies (Glass et al, 2005; Etherington et al, 2007). In contrast, in my study the greater rise in markers of tubular injury after repeated antibiotic treatment was associated with a significant rise in serum creatinine. This confirms that the urinary enzymes and proteins employed here serve as sensitive markers of early tubular injury and that ultimately sufficient tubular impairment accumulates to produce clinically overt functional derangement. This direct temporal association between aminoglycoside-induced enzymuria and higher serum creatinine levels has not been previously reported.

Of course, colistin, also a tubulotoxin, may be partly responsible for the observed changes in urinary assays (Falagas et al, 2005; Falagas and Kasiakou, 2006; Etherington et al, 2007). However, tobramycin produced much bigger urinary NAG

signals than colistin in a previous study (8-fold *vs* 2-fold rise respectively) (Etherington et al, 2007). Therefore most of the changes observed in my experiment are likely secondary to the aminoglycoside. Furthermore, colistin was used in both consecutive exposures; this should minimize confounding effects of the second antibiotic.

Based on these results, it would seem sensible, in the management of frequent exacerbators requiring a high burden of antibiotic therapy, that alternative anti-pseudomonal antibiotic classes are rotated incorporating "aminoglycoside breaks"/ "aminoglycoside holidays", whilst maintaining surveillance for aminoglycoside- and other antibiotic-related nephrotoxicity.

7.5 TABLES

Table 7.5.1

Baseline characteristics on day 0 of two consecutive admissions (n=40)

	First treatment			Second treatment		
	mean	SD	range	mean	SD	range
BMI (kg/m^2)	21.3	2.5	16.6-26.2	21.6	2.2	17.1-25.8
Blood urea (mmol/l)	7.2	2.2	3.8-10.6	7.0	2.5	3.7-10.7
Serum creatinine (umol/l)	82.5	14.1	59.0-112.0	84.4	18.0	56.6-120.2
Creatinine clearance (ml/min)	105.4	31.8	49.9-153.2	99.7	31.2	51.0-154.0

Table 7.5.2

Changes in urinary enzymes and proteins with the first IV antibiotic treatment

(n=40)

Data presented as mean and (SD)

Urinary marker	Day 0	Day 14	P value
NAG (u/mmol)	0.41 ((0.57)	3.27 (2.32	<0.0001
AAP (u/mmol)	0.83 (0.50)	25.42 (21.27)	<0.0001
β2M (mcg/mmol)	20.31 (11.98)	128.12 (112.19)	<0.0001

Table 7.5.3

Changes in urinary enzymes and proteins with treatment

Group 1: n= 25, group 2: n = 15. Data presented as mean and (SD)

marker	group	Treatment 1			Treatment 2		
		Day 0	Day 14	change	Day 0	Day 14	change
NAG (u/mmol)	1	0.41 (0.61)	3.35 (2.51)	2.94 (2.44)	0.33 (0.54)	3.09 (1.95)	2.76 (1.94)
	2	0.40 (0.52)	3.14 (2.02)	2.73 (1.67)	1.35 (1.20)	5.81 (2.52)	4.45 (2.18)
P value *vs* treatment 1					0.0003		0.011
AAP (u/mmol)	1	0.78 (0.53)	26.51 (27.10)	26.09 (25.32)	0.66 (0.43)	24.29 (17.56)	23.63 (17.60)
	2	0.91 (0.44)	22.99 (12.07)	22.08 (11.92)	7.09 (4.84)	42.44 (19.71)	35.35 (16.80)
P value *vs* treatment 1					0.0002		0.010
β2M (mcg/mmol)	1	19.76 (12.61)	130.01 (123.39)	110.25 (117.73)	21.14 (8.98)	134.09 (100.61)	112.95 (99.53)
	2	21.22 (11.20)	124.98 (94.60)	103.76 (92.75)	55.29 (30.47)	226.36 (136.66)	171.07 (135.43)
P value *vs* treatment 1					0.0021		0.189

Table 7.5.4

Patient group allocation prior to the second aminoglycoside challenge

Group 1 denotes complete *vs* incomplete (group 2) recovery of tubular markers prior to aminoglycoside *re-exposure*

	Group 1	**Group 2**
N	25	15
Mean interval between treatments in weeks (range)	14 (11-16)	5 (3-7)

7.6 FIGURES

Figure 7.6.1

Baseline characteristics on day 0 of two consecutive admissions (n=40). Whiskers represent range

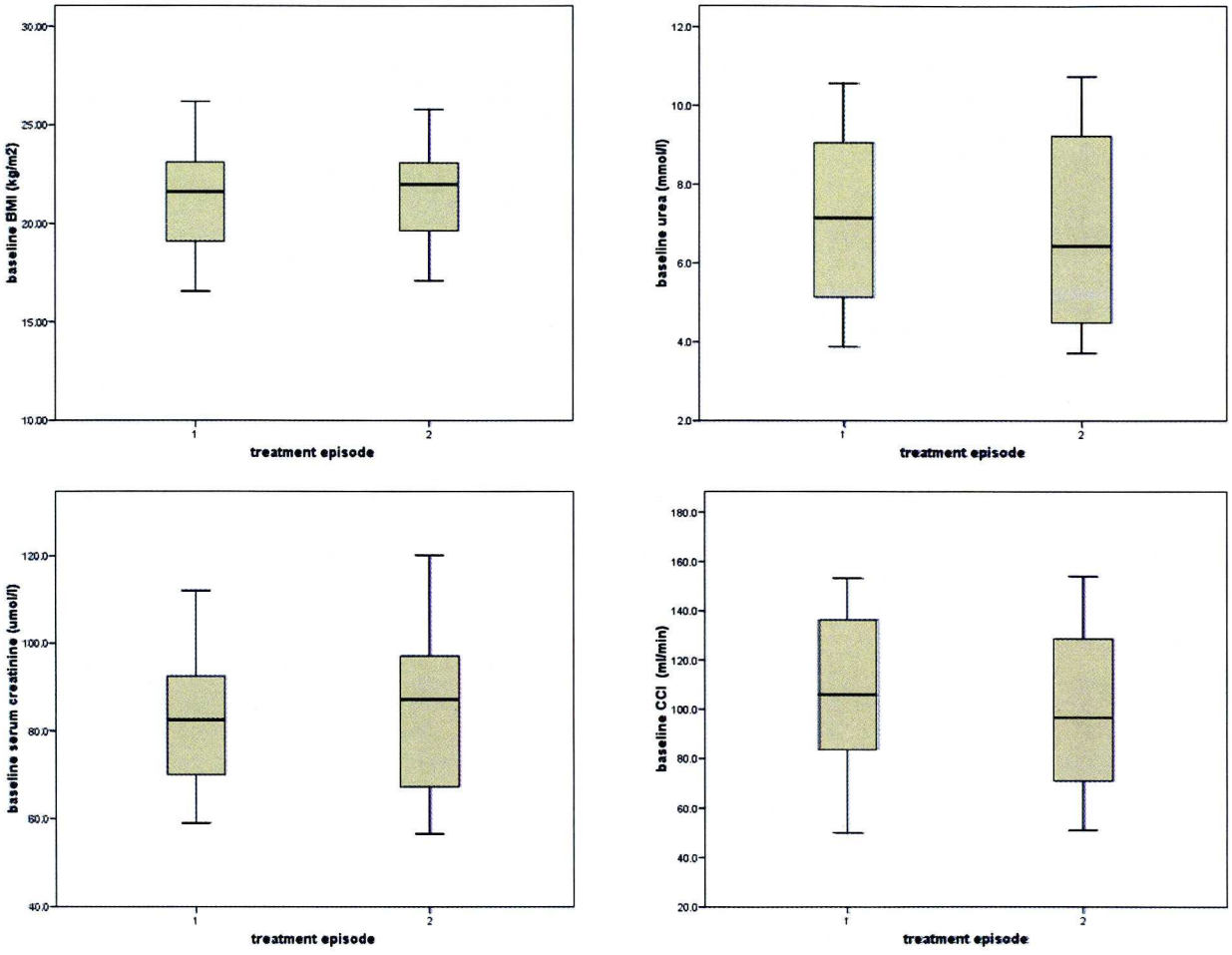


Figure 7.6.2

Changes in blood urea with treatment

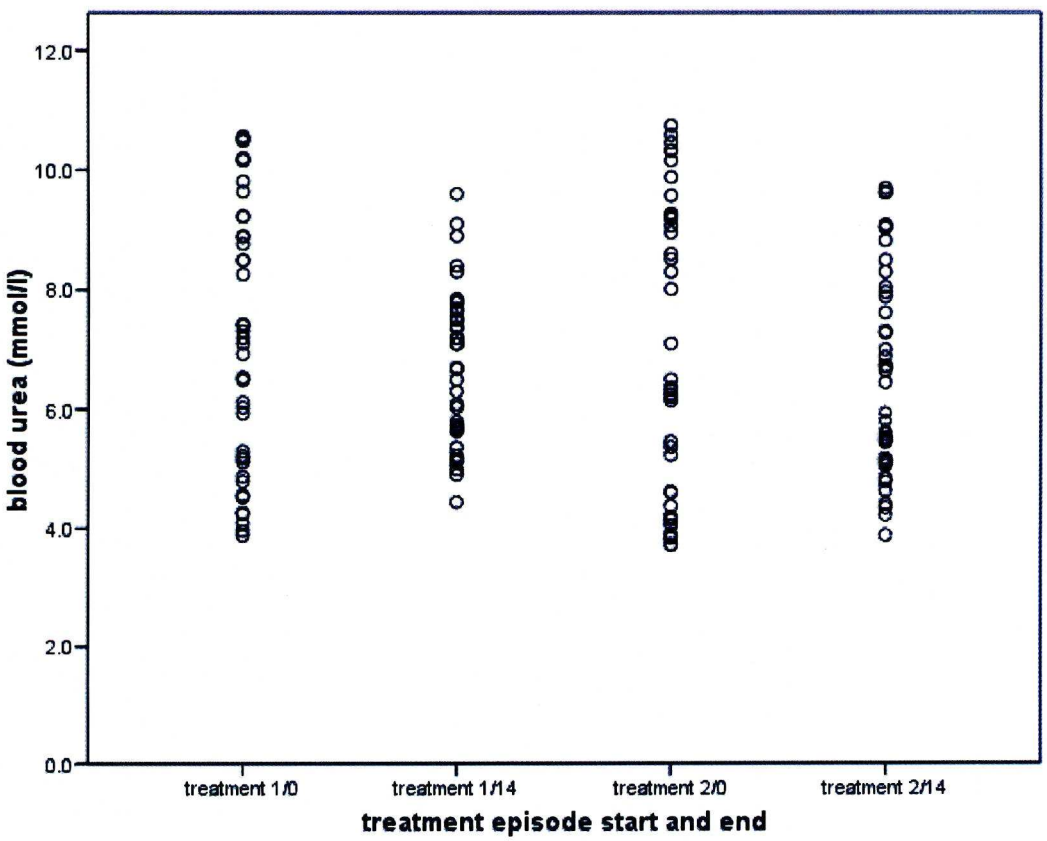
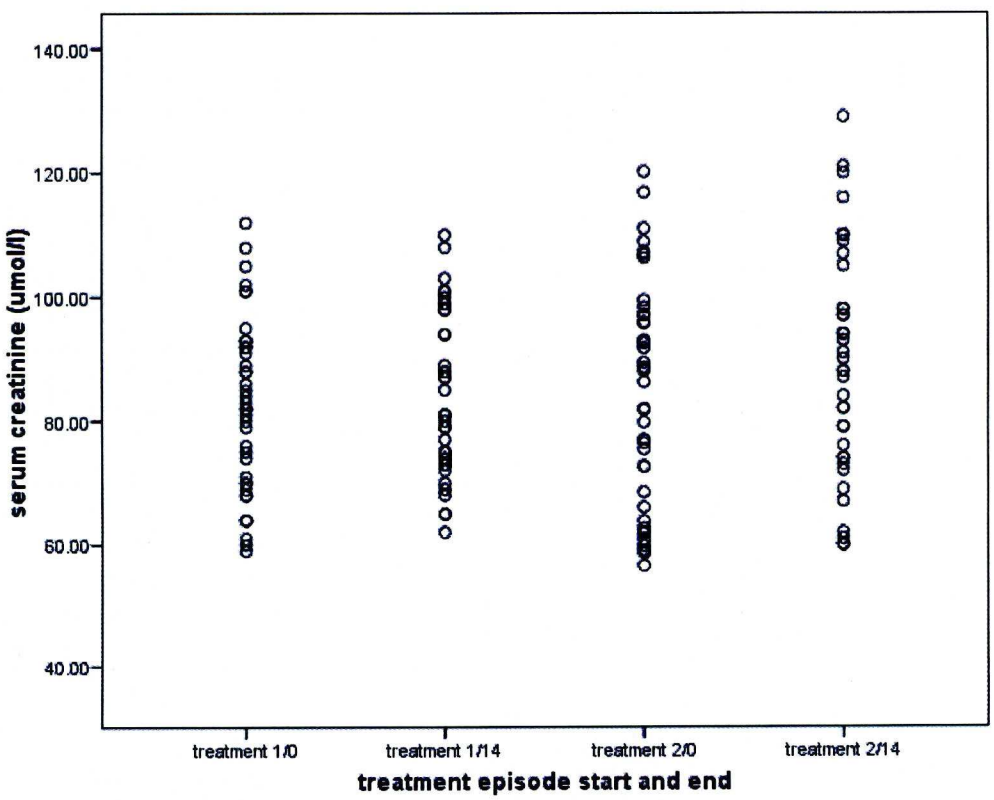


Figure 7.6.3

Changes in serum creatinine

a. by treatment



b. by group and treatment

Dotted lines represent the local laboratory reference range

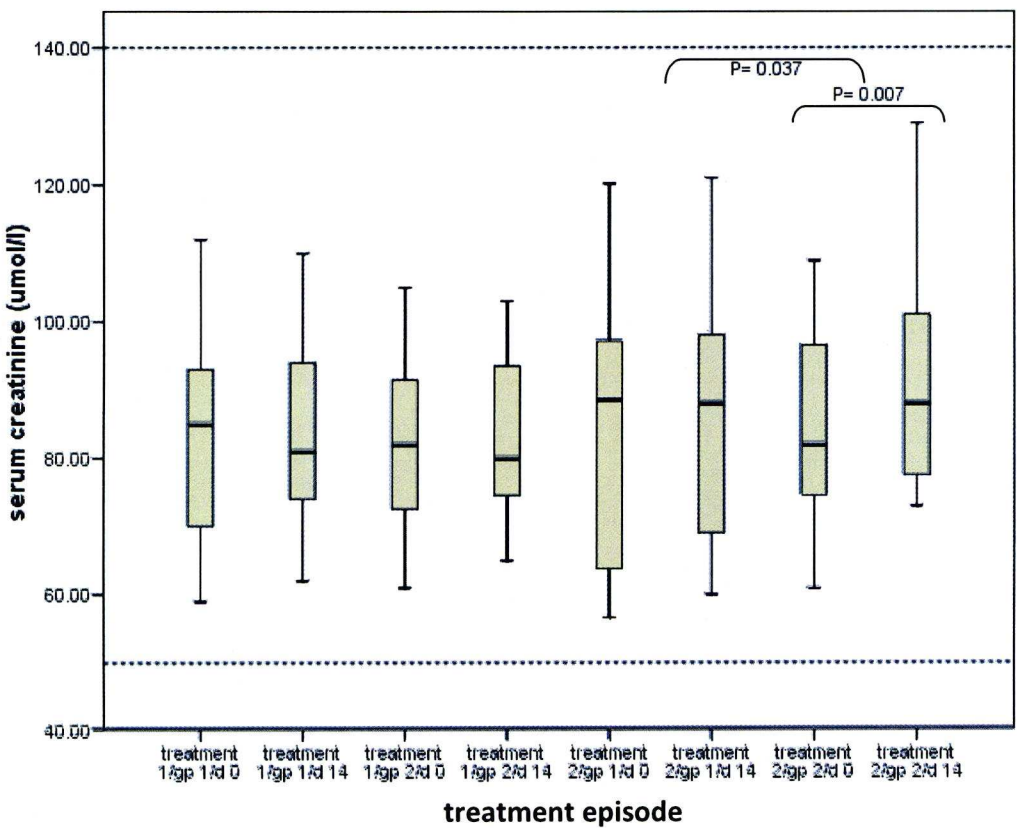


Figure 7.6.4

Changes in urinary NAG with treatment

Group 1: n= 25, group 2: n = 15

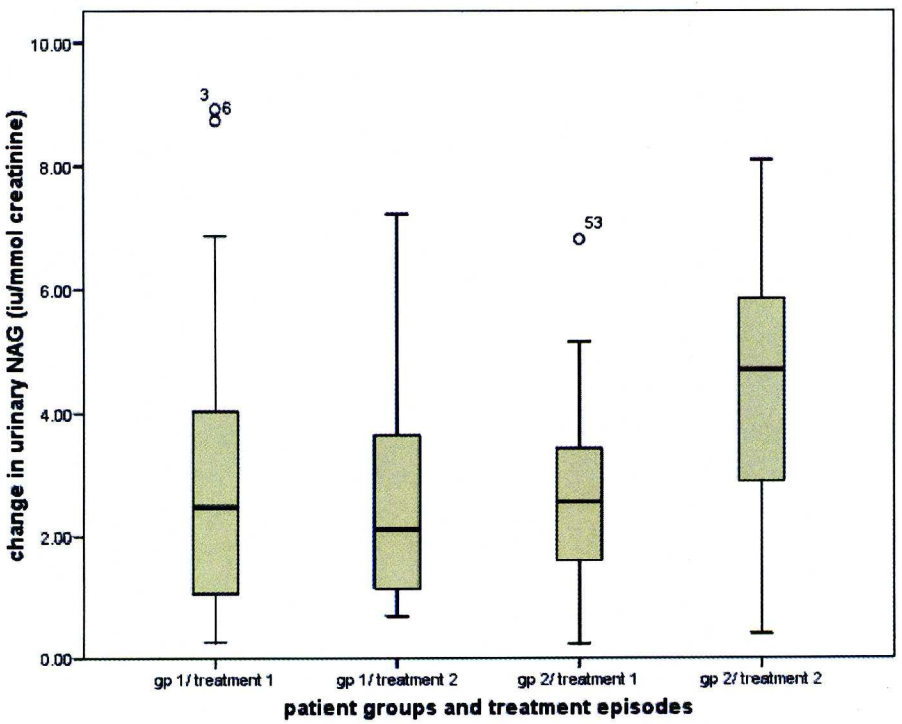


Figure 7.6.5

Changes in urinary AAP with treatment

Group 1: n= 25, group 2: n = 15

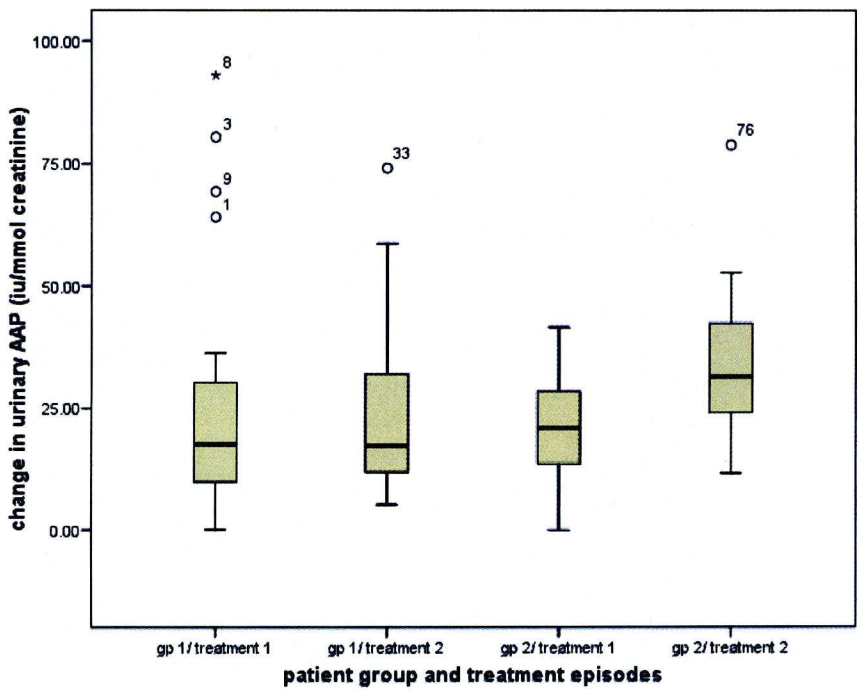


Figure 7.6.6

Changes in urinary β2M with treatment

Group 1: n= 25, group 2: n = 15

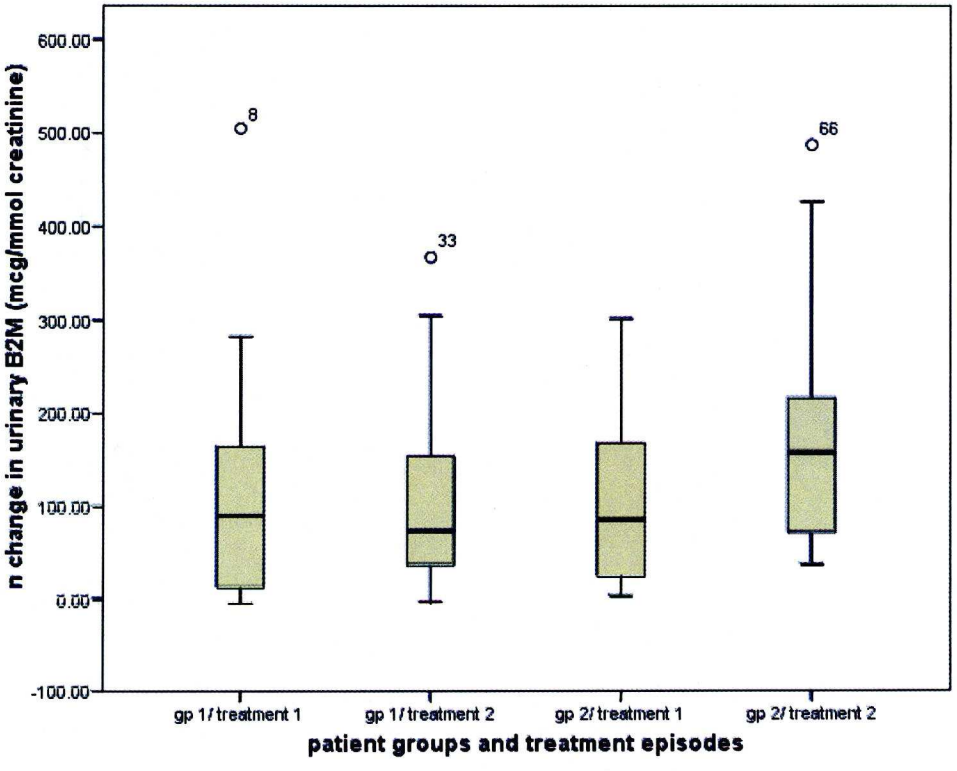


Figure 7.6.7

Correlation between trends in urinary markers after the first treatment

a) NAG vs AAP

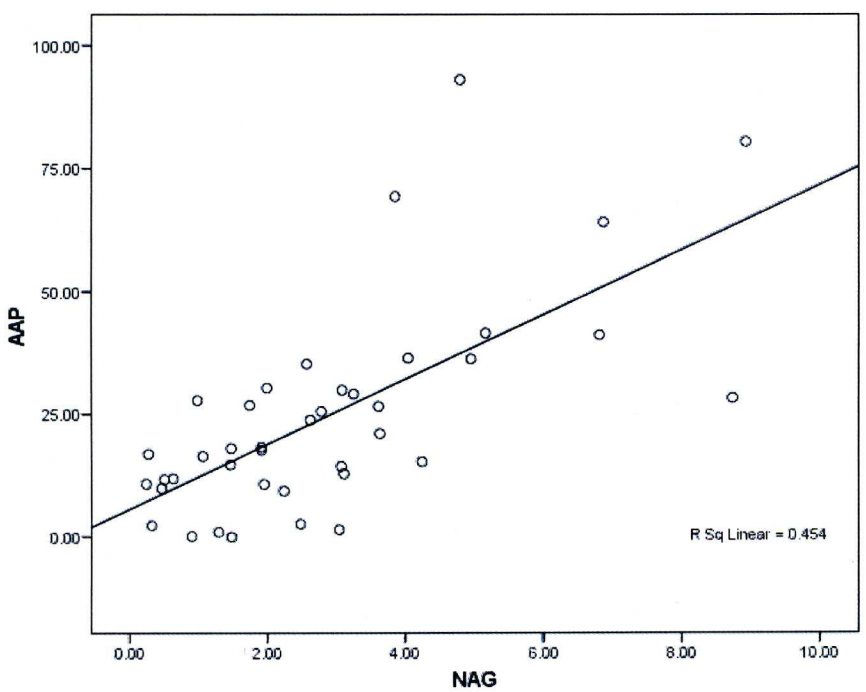


Figure 7.6.7 continued

B) NAG vs β2M

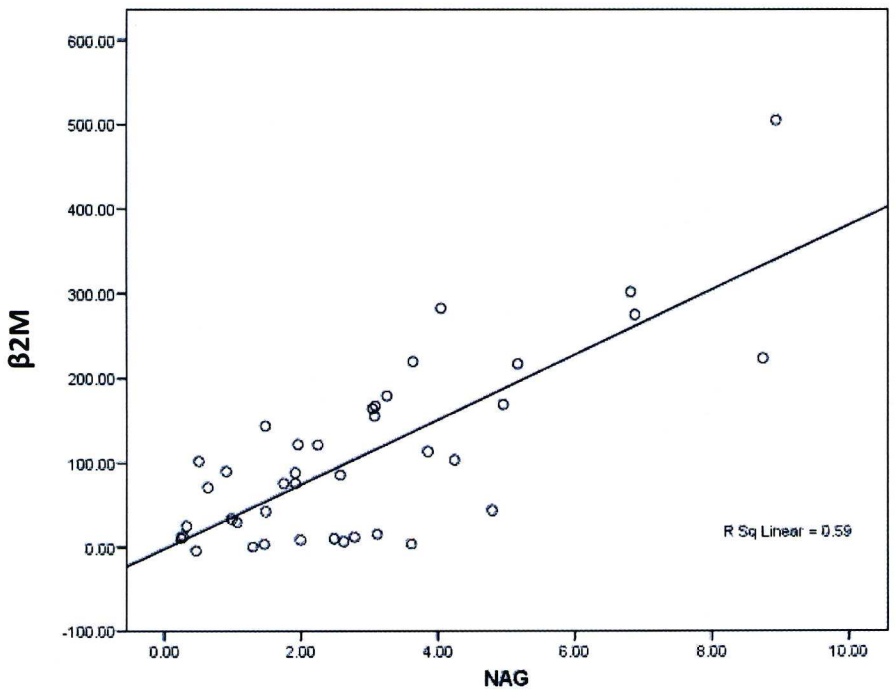


Figure 7.6.7 continued

C) AAP vs β2M

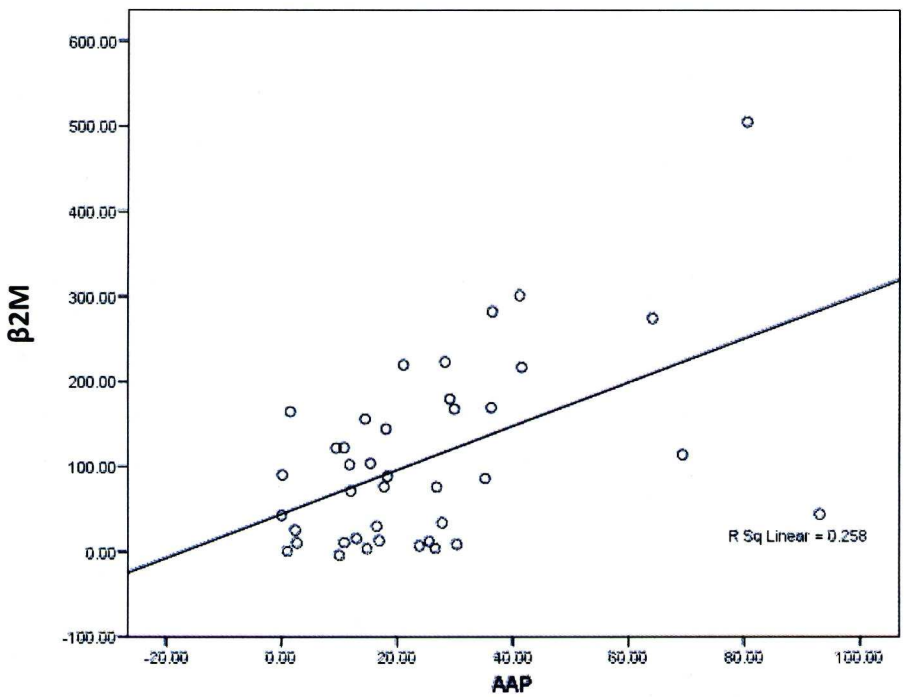


Figure 7.6.8

Time to recovery of renal tubular markers after the first antibiotic treatment. Bars represent individual patients in whom predefined "complete recovery" thresholds were met.

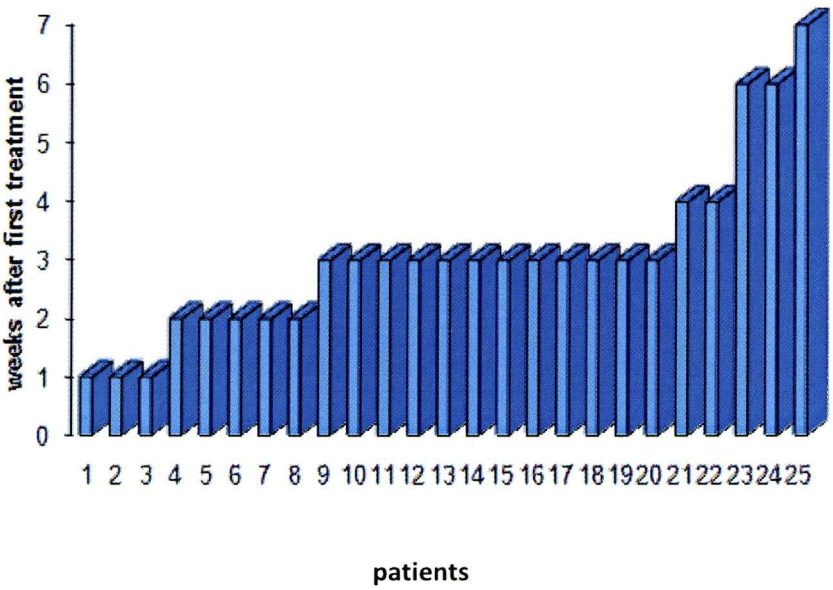
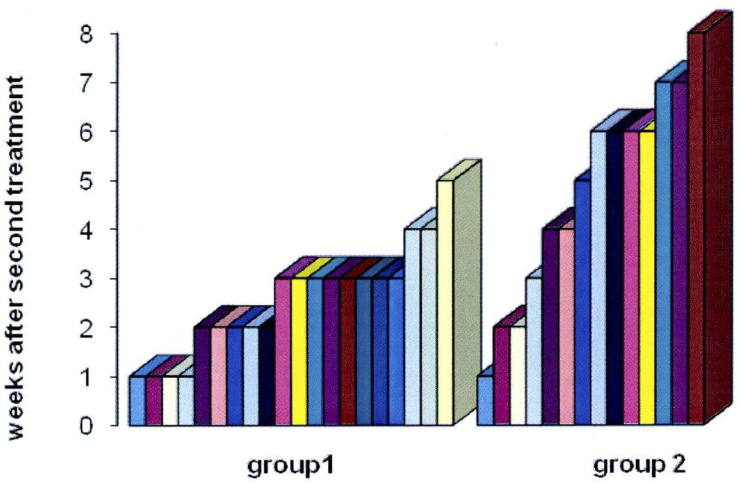


Figure 7.6.9

Time to recovery of renal tubular markers after the second antibiotic treatment.

Bars represent individual patients for whom complete follow up data were available (n=34). Group 1: n= 20; Group 2: n=14.



CHAPTER EIGHT

Pilot cross over study of high dose nebulised *vs.* intravenous tobramycin in combination treatment of pulmonary exacerbations in adult CF patients

8.1 INTRODUCTION

Aminoglycosides are powerful antibiotics frequently used intravenously to treat exacerbations of *P. aeruginosa* airway infection in CF patients. With the emergence and increasing prevalence of multi-drug resistant strains (Scott and Pitt, 2004), aminoglycosides are likely to assume a more central role in the management of CF lung disease. Indeed, the commonest transmissible *P. aeruginosa* strain in the UK, the Liverpool epidemic strain (LES) (Scott and Pitt, 2004) is usually only predictably sensitive to tobramycin and colistin (Mirakhur et al, 2003). However, aminoglycosides are renal tubular toxins and their IV use has been linked with reports of acute renal failure in CF children (Kovesi et al, 1998; Drew et al, 2003) and adults (Chapter 5, Bertenshaw et al, 2007; Smyth et al, 2008) . I have also shown that in the long term, cumulative IV aminoglycoside dosing can impair renal function as manifest by reduced glomerular filtration rate (Chapter 4).

In contrast, aerosolised delivery of these antibiotics achieves higher sputum concentrations, while potentially minimising the risks of nephrotoxicity associated with systemic delivery (LiPuma, 2001). Although Ring *et al* (Ring et al, 1998) demonstrated a positive correlation between markers of renal injury and the cumulative dose of inhaled gentamicin, the effect of inhaled tobramycin therapy has only been studied using low doses where no renal tubular adverse effects occurred (Steinkamp et al, 1986). To investigate these aspects further, using a randomised crossover trial design I carried out a pilot study comparing the impact of high dose nebulised tobramycin with IV tobramycin on lung and kidney function in *P. aeruginosa* exacerbations in patients with CF.

8.2 PATIENTS AND METHODS

8.2.1 Study population

I recruited 20 CF patients (mean [SD] age: 22.1 [6.9] years, forced expired volume in 1 second (FEV1): 64.7 [21.9] % predicted, body mass index (BMI): 20.2 [3.5] kg/m^2, 11 males), chronically infected with multiresistant morphotypes of LES *P. aeruginosa* who were admitted to the Liverpool Adult CF Unit with pulmonary exacerbations. Chronic infection was defined as 3 or more positive sputum cultures within the previous 12 months (Cystic Fibrosis Trust Control of Infection Group 2001), and multi-resistance as resistance to at least 2 of the 3 standard antipseudomonal antibiotic classes (Cystic Fibrosis Trust Antibiotic Group 2002). An exacerbation was defined as worsening respiratory symptoms accompanied by spirometric reductions (Rosenfeld et al, 2001a). Patients with known hypersensitivity to aminoglycosides/colistin, significant haemoptysis or new radiographic changes, a history of *Burkholderia cepacia* complex isolation in the preceding 12 months, and those who had received any aminoglycoside (IV or nebulised) therapy during the previous 3 months or any additional antipseudomonal antibiotic in the 2 weeks prior to randomisation were excluded. Six had CF-related diabetes at enrolment but no new cases of diabetes were diagnosed in the remainder over the study period. Changes to maintenance therapy were not permitted within 14 days of the exacerbations. None had fever or engaged in physical exercise for 48 hours prior to recruitment. Patients gave written informed consent and the local ethics committee approved the study protocol.

8.2.2 Study design

Patients were randomised to receive 14 days of either preservative-free pH-adjusted tobramycin nebuliser solution (TNS) (TOBI®, 300 mg/5ml, Novartis, Surrey, UK) or IV tobramycin (80 mg/2ml, Mayne Pharma Plc, Warwickshire, UK).

TNS was administered using a Pari LC Plus™ jet nebuliser (Pari Medical Ltd, West Byfleet, UK) and a Porta-Neb® compressor (Medic-Aid Ltd, West Sussex, UK) at a dose of 300mg bd. Although there are no established reference values for serum tobramycin concentration during nebulised therapy, we measured tobramycin levels one hour after TNS dosing on days 2 and 14.

IV tobramycin was given at the standard mean daily dose of 8.2 mg/kg [SD 1.5] in 2 – 3 divided doses. According to local protocols, aminoglycoside levels were measured pre and one hour after the fourth administration and the dose adjusted to achieve a trough level of < 2.0 mg/l and a peak level of 6-10 mg/l. Subsequently, levels were measured as needed to ensure therapeutic serum concentrations.

Sputum isolates were only susceptible to colistin and tobramycin *in vitro*. Therefore, all patients also received IV colistimethate sodium (Colomycin® injection, Forest Laboratories Ltd, Kent, UK) 2 megaunits tid, in line with the UK CF Trust guidelines advocating a minimum of 2 anti-pseudomonal antibiotics to treat pulmonary exacerbations (Cystic Fibrosis Trust Antibiotic Group 2002).

When each patient was re-hospitalised at a later date with a second exacerbation, they received the alternative form of tobramycin and the same dose of IV colomycin (figure 8.6.1).

The mean interval between admissions was 6.6 months [SD 4.1; range 3 – 20]. Allowing for age change, baseline patient characteristics were similar and the remainder of their therapy was otherwise identical in the 2 inpatient episodes (azithromycin: 8 patients, NSAIDS: 2, rhDNase: 8, IV aminophylline: 4, subcutaneous bricanyl: 2).

8.2.3 Outcome measures

8.2.3.1 Efficacy

Clinical evaluation and spirometry were performed and sputum samples obtained for quantitative microbiology on admission (day 0) and on day 14 (12 hours after the previous evening nebulised/IV dose). The primary efficacy end point of the study was the mean change in FEV1% predicted from baseline to day 14. Changes in forced vital capacity (FVC) % predicted, peripheral blood white cell count (WCC) and C-reactive protein (CRP) were also noted. Impact on sputum *P. aeruginosa* density and time to next exacerbation were compared between the two treatment strategies. Patients rated their overall satisfaction with improvement during treatment on a visual analogue scale (VAS: 0 = not satisfied – 10 = completely satisfied), where for repeat testing patients where blinded to their previous responses.

8.2.3.2 Safety

Safety endpoints included adverse events, incidence of TNS-induced acute bronchospam (evaluated by measuring FEV1 before and 30 min after the first TNS dose and defined as >10% drop in FEV1), serum tobramycin concentrations and changes in renal function. The latter was measured before and at the end of 2 week treatment and included serum creatinine (SCr) and magnesium (Mg^{2+}) assays, 24 hour urine collection for measurement of creatinine clearance (CCl) and total proteinuria, and urinary levels of the proximal tubular enzyme N-acetyl-β-D-glucosaminidase (NAG).

8.2.4 Laboratory methods

Sputum samples were transferred on wet ice to the microbiology laboratory and processed within 48 hours of collection. Samples were cultured at the beginning and end of treatment to assess sputum *P. aeruginosa* density (log_{10} colony forming units (cfu)/ml). Quantification of Pseudomonas counts in sputum was performed according to methods previously described by Burns and coworkers (Burns et al, 1998). Sputa were homogenised by the mixing of equal volumes of sputum and sputasol (Oxoid Ltd, Basingstoke, UK) in screw-topped plastic universal containers which were incubated at room temperature for 20 minutes with vortexing. 100 µl aliquot of each sample was then plated on a MacConkey (Bioconnections, Leeds, UK) and a pseudomonas selective agar plate (Pseudomonas agar base enriched with pseudomonas CN selective supplement; Oxoid Ltd, Basingstoke, UK). The plates were incubated in air at 37°C for 48 hours. Subsequently the colonies grown were counted using the Stuart Scientific Colony Counter.

For measurements of serum tobramycin levels, serum and urine creatinine concentrations, calculation of mCCls, quantitation of 24-hour urinary protein elimination and urinary NAG levels please refer to chapter 3 (General laboratory methods). The effects of varied urine concentration were minimised by expressing NAG level as a ratio of urine creatinine. Results are reported as units/mmol creatinine.

8.2.5 Statistical analysis

Baseline demographics were compared using a paired t-test or chi-squared test. Within group changes in pulmonary function, blood, sputum and urine tests were assessed using a paired t-test (parametric data) or the Mann-Whitney U test (non parametric data). Treatment effect, 95% CI, and P values were analysed using a

paired t-test or Wilcoxon rank sum test as appropriate. Comparison of time to next exacerbation was conducted with Kaplan Meier survival curves and a log rank test. Results are presented as means (standard deviation (SD)), unless otherwise stated. Analysis was conducted with SPSS for windows version 15.0.

8.3 RESULTS

8.3.1 Treatment efficacy

All 40 treatment episodes concluded satisfactorily and none failed to complete therapy. The mean (SD) 14 day improvement in FEV1 % predicted was similar for both treatments: 19.9% (11.3) with TNS and 16.4% (8.5) with IV tobramycin. Mean difference between treatments was 3.6 (95% CI -9.7 to 2.6, P=0.26). Figure 8.6.2 shows individual patient FEV1 data. FVC, WCC and CRP displayed similar patterns (table 8.5.1).

8.3.2 Effect on sputum *P. aeruginosa* density

Sputum before and after each treatment (i.e. 4 samples [2 pairs] per patient) were submitted by 15 patients; sample quality in 2 prevented further quantitative analysis. In the remaining 13 patients, baseline sputum *P. aeruginosa* density was similar before the 2 treatments [TNS: 7.31 $\log_{10}$ cfu/ml (3.11) *vs* IV tobramycin 6.92 (1.93), P=0.68]. Although both treatments significantly reduced sputum *P. aeruginosa* counts [-2.81 $\log_{10}$ cfu/ml (0.97), p<0.001 *vs* -1.56 (1.31), P<0.05], treatment effect was greater with TNS (mean difference between treatments -1.25 $\log_{10}$ cfu/ml, 95% CI -2.67 to -0.33, P<0.05). Treatment-emergent superinfection with new pathogens was not noted in any of these episodes.

8.3.3 Time to next exacerbation

TNS therapy significantly prolonged the time to next exacerbation requiring hospitalisation. The interval between 2 consecutive hospital admissions was 8.9 (SD 4.7) and 4.3 (SD 1.3) months for patients treated initially with TNS and IV tobramycin respectively, χ^2 test = 12.39; HR 3.64, 95% CI 2.62-29.6, P<0.001 (figure 8.6.3).

8.3.4 Patient appraisal of therapy

VAS scores at the end of therapy were a mean of 8.1 (1.3) for TNS compared with 8.5 (1.1) for IV therapy, for an observed difference in the means between treatments of 0.4 (95% CI -0.34 to 1.14, P=0.29), suggesting that patients were equally satisfied with the improvement achieved on either treatment.

8.3.5 Safety

8.3.5.1 Adverse events (AEs)

There were no AEs warranting withdrawal of therapy. The incidence of AEs was comparable during TNS and IV tobramycin therapy. Treatment-emergent AEs were recorded in 11 (55%) and 9 (45%) of TNS and IV tobramycin treatment episodes respectively. Hoarseness was the most common TNS-related AE compared with increased cough with IV therapy (table 8.5.2). Acute bronchospasm was not observed in any of the 20 TNS treatment episodes, and neither treatment induced acute chest tightness necessitating rescue inhaled or nebulised bronchodilator therapy. One patient developed minor haemoptysis 2 days after starting TNS: dosing was interrupted for 3 days until the haemoptysis resolved, and subsequently recommenced without further complications.

8.3.5.2 Serum tobramycin levels

No toxic drug levels were recorded on either treatment. For the IV route, mean trough and peak serum tobramycin levels measured immediately before and 1 hour after the 4th dose (on day 2) were 1.2 (0.3) and 7.3 (1.5) mg/l respectively, and paired tobramycin assays (median 2, range 1-4 per patient) showed trough and peak levels of 1.4 (0.6) and 7.6 (1.4) mg/l respectively. Tobramycin concentrations one hour after TNS on day 2 and day 14 were 1.4 (0.4) and 1.2 (0.7) mg/l respectively.

8.3.5.3 Effects on renal function

Prior to treatment, urine dipstick was negative in all patients and none had pathological proteinuria. All had normal renal ultrasound scans at the preceding annual review and none had received organ transplantation.

Mean SCr was similar at baseline [TNS *vs* IV tobramycin: 82 (11.4) micromol/l *vs* 83.2 (12.6), P=0.75] and did not change significantly on either treatment. Mean % change from baseline in SCr was 4.3% (9.4) with TNS *vs* 3.6% (10.4) with IV tobramycin, for a mean difference between treatments of 0.7, 95% CI: -6.8 to 5.4, P=0.83. In all patients, SCr remained within the normal range (figure 8.6.4).

Similarly, Serum Mg^{2+} did not change significantly during either treatment. Mean change was -0.07 mmol/l (0.14) for TNS *vs* -0.09 mmol/l (0.23) for IV tobramycin, with a mean difference of 0.02, 95% CI -0.09 to 0.14, P=0.74. Three IV treatment episodes but none of the TNS episodes were complicated by mild hypomagnesaemia (0.60 - 0.69 mmo/l), without associated symptoms.

CCl was also similar at baseline [TNS *vs* IV tobramycin: 67.9 (23.8) ml/min *vs* 71.6 (29.1), P=0.66]. Significant improvements in CCl were noted at the completion of both treatments (figure 8.6.5). With TNS, mean % change from baseline in CCl was 23.9% (48.4) [P<0.05] compared with 26.1% (35.0) [P<0.05] with IV tobramycin, for a mean difference between treatments of 2.2%, 95% CI: -24.0 to 28.4, P=0.87.

However, IV tobramycin was associated with greater urinary protein leak than TNS [mean difference 0.58, 95% CI 0.30 to 0.87, p<0.001), figure 8.6.6]. Urinary levels of NAG were log normally distributed and rose significantly on IV tobramycin (mean change from baseline: $\log_{10}$ 0.74 (0.44) iu/mmol) but not TNS ($\log_{10}$ 0.02 (0.51) iu/mmol) with a mean difference between treatments of 0.72 (95% CI 0.37 to 1.07, P <0.001).

8.4 DISCUSSION

To my knowledge, this pilot study is the first to assess the utility of an aerosolised aminoglycoside as an adjunct therapy in acute exacerbations of *P. aeruginosa* infection in CF patients where quantitative microbiological measurements were performed. The main limitation of this study is that it was not powered to demonstrate treatment equivalence. Nevertheless, the results suggest that, when combined with a second antipseudomonal antibiotic, high dose TNS (TOBI®, Novartis, Surrey, UK) is as effective as IV tobramycin, in the treatment of acute pulmonary exacerbations in CF, with the added advantages of delaying subsequent exacerbations and less immediate renal tubular cell injury.

Previous work comparing different IV treatment regimes for tobramycin (Smyth et al, 2005) suggested that studying 130 patients would give 80% power to show treatment equivalence. Conversely, routine data from the Liverpool adult CF unit indicate a standard deviation of 10% for the change in FEV1 % predicted over 14

days antibiotic treatment. Combining these calculations, 80 patients would have to be recruited to achieve the same power level (80%) assuming that a difference of 4% or less in the mean change in FEV1 % predicted is tolerated between equivalent regimens. This invites a multicentre study to confirm the findings of this pilot study

As expected, eradication of *P. aeruginosa* was not observed in any of the treatment episodes evaluated microbiologically, since successful treatment of an exacerbation in chronic airway infection does not depend on the eradication of the organisms but a reduction in bacterial density (Smith et al, 1988; Regelmann et al, 1990). The superior efficacy of TNS over IV tobramycin in this respect (a reduction in *P. aeruginosa* counts of 2.81 *vs* 1.56 $\log_{10}$ cfu/ml respectively) could partly explain the longer time to next exacerbation noted after TNS therapy. These two observations taken together also reassure me that the clinical improvement noted in the nebuliser arm was not simply related to the concomitant use of IV colistin. Indeed, it has already been shown that IV colistin used as monotherapy in treating CF exacerbations is less effective than in combination (Conway et al, 1997). In keeping with accepted CF practice (Cystic Fibrosis Trust Antibiotic Group 2002), I ensured that all exacerbations were treated with at least two relevant antipseudomonal antibiotics. Furthermore, I controlled the effect of the second antipseudomonal antibiotic by ensuring that the same one was used throughout the study. To eliminate any confounding effects of other antipseudomonal therapy, I excluded patients who received IV or nebulised antibiotics up to 3 months and oral antibiotics up to 2 weeks prior to randomisation and no nebulised antibiotics, except those per study protocol, were permitted throughout the study period.

The primary rationale for the use of aerosolised antibiotics in chronic bronchial sepsis is improved targeted delivery to the site of infection whilst minimising systemic bioavailability and associated toxicities (Mendelman et al, 1985; LiPuma, 2001; Moss, 2001). TNS (TOBI®) has excellent lung deposition with mean peak

sputum concentrations up to 15 times higher than with IV tobramycin (Eisenberg et al, 1997; Weber et al, 1997; LiPuma, 2001). This is very relevant to aminoglycosides since they display concentration dependent bacterial killing (LiPuma, 2001). It may explain the greater impact from the inhaled antibiotic on sputum bacterial load noted in the present study, which has not previously been reported in comparison with IV administration in the setting of an acute CF exacerbation. The addition of TNS to oral ciprofloxacin in pulmonary exacerbations in non-CF bronchiectasis patients chronically infected with *P. aeruginosa* has already been shown to enhance the reduction in bacterial load (Bilton et al, 2006): if similar results were reproduced in CF, such a combination may reduce the requirement for hospitalisation and IV therapy.

The possibility that this observation was biased by the effect of antibiotic carryover in the respiratory secretions cannot be entirely ruled out. However, sputum bacterial densities remained significantly reduced one week after completion of TNS dosing in non-CF bronchiectasis (Bilton et al, 2006) and failure to culture *P. aeruginosa* at the end of 28 day TNS treatment in paediatric CF patients has been shown not to be related to residual tobramycin in bronchial lavage fluid (Gibson et al, 2003b). Furthermore, to minimise this effect, I collected sputa no earlier than 12 hours after the last TNS dose.

I did not measure tobramycin MIC_{50} or MIC_{90} before or after treatment for several reasons. Firstly, the conventional definition of drug resistance and sensitivity (ie, MIC breakpoint of 8-16 mcg/ml) as determined by parenteral tobramycin therapy may not be applicable to TNS, and indeed clinical improvement with TOBI® has been shown to be independent of conventional MIC category (Ramsey et al, 1999; Moss, 2001). Secondly, although long-term intermittent TNS use can confer an incremental increase in resistance among *P. aeruginosa* isolates (Burns et al, 1999; Ramsey et al, 1999; LiPuma, 2001), this is not seen when the therapy is shorter than

28 days (LiPuma, 2001) and any acquired resistance recovers when the antibiotic pressure is removed (LiPuma, 2001). Indeed, a return toward drug susceptibility after each of the 1-month off-drug intervals was observed in the US inhaled tobramycin studies (Burns et al, 1999; Ramsey et al, 1999; LiPuma, 2001). Therefore, treatment limited to 14 days only, followed by longer off-treatment gaps in my study (mean 6.6 months) suggests that TNS is unlikely to render *P. aeruginosa* isolates more resistant and cannot explain our observed differences in microbiological effect. Furthermore, in a non-CF bronchiectasis study (Bilton et al, 2006), only one of 26 developed a resistant *P. aeruginosa* after TNS treatment. I acknowledge that the use of high range E-test strips would have been helpful to clarify the increase or maintenance of tobramycin MICs between treatments (Morosini et al, 2005).

Substantial variations of peak serum levels and areas under the curve have been observed in CF patients on inhaled tobramycin therapy (Eisenberg et al, 1997; Touw et al, 1997; Geller et al, 2002), but systemic absorption is generally minimal and toxicity has not been reported in randomised controlled trials. Systemic bio-availability of 11.7% with a median serum concentration one hour after inhalation of 0.98 mg/l and no higher than 2 mg/l are representative figures in stable CF patients (Touw et al, 1997; Geller et al, 2002; Gibson et al, 2003b). There is a theoretical possibility that the delivery of aerosolised aminoglycoside to the acutely inflamed and hyperaemic airways during an exacerbation may increase the risk of bronchospasm (Alothman et al, 2002) or enhance local drug absorption and its systemic bioavailability. However, in this study acute bronchoconstriction was not recorded in any of the 20 TNS episodes, and my 1 hour assays (mean 1.4 mg/l) suggest that systemic absorption of the aerosol was not augmented during infective exacerbations. Furthermore, in the TNS arm serum tobramycin levels were comparable at the beginning and end of the 14 day treatment period and were similar to concentrations previously reported in studies of maintenance TNS

therapy in CF and non-CF bronchiectasis (Ramsey et al, 1999; Geller et al, 2002; Scheinberg and Shore, 2005; Bilton et al, 2006).

I have previously shown that repeated IV aminoglycoside exposure in CF patients causes renal impairment (chapter 4) and IV aminoglycosides, particularly gentamicin, were implicated in a recent survey of acute renal failure in CF in the UK (Smyth et al, 2008). Antibiotic choice is becoming increasingly limited in an era characterised by rising antibiotic resistance, and the frequent need for aminoglycosides prompted a search for renal-sparing alternative therapy. A recent report suggested that once daily IV tobramycin in the treatment of CF pulmonary exacerbations might be less nephrotoxic in children compared with thrice daily regimens (Smyth et al, 2005). However, a similar protective effect was not seen in adults, who displayed a higher serum creatinine after once daily therapy and the study did not have sufficient power to exclude greater nephrotoxicity in the adult subgroup (Smyth et al, 2005). The same authors have since demonstrated reduced tobramycin elimination rate on once daily therapy, postulating early renal damage caused by the higher tobramycin boluses that could not be detected by the biochemical assays they used (Touw et al, 2007). The Liverpool adult CF unit has therefore not adopted once daily aminoglycoside therapy and consequently it was not used in the control arm of the present cross over study.

An inherent difficulty in this subject is how best to survey for acute drug related renal injury. Serum creatinine is not sufficiently sensitive to detect early changes in renal function because its parabolic relationship with creatinine clearance (CCl) may fail to reveal significant changes in GFR (Baker, 2002). In keeping with this, serum creatinine in our study did not alter despite a 25% increase in CCl in both treatment arms. Conversely, whilst CCl is helpful in the long-term monitoring of renal function in clinically stable patients (chapter 4), its use to detect subclinical drug-induced acute renal toxicity in acute pulmonary exacerbations may be limited by

interventions given to reverse the hypercatabolic volume-depleted state typical of the condition (Baker, 2002).

In contrast, measurement of proteinuria and enzymuria may serve as sensitive indices of the early stages of renal damage (Steinkamp et al, 1986; Ring et al, 1998; Mingeot-Leclercq and Tulkens, 1999). Filtered aminoglycosides enter the proximal tubular epithelium by endocytosis where they adhere to the lysosomal membrane, causing it to leak and rupture (Mingeot-Leclercq and Tulkens, 1999). Even therapeutic doses of aminoglycosides can cause proximal tubular injury resulting in leucocyturia, proteinuria, and elevated urinary excretion of some tubular enzymes (Mingeot-Leclercq and Tulkens, 1999), where their release is proportional to the damage caused, long before conventional assays become deranged (Steinkamp et al, 1986; Ring et al, 1998; Mingeot-Leclercq and Tulkens, 1999). NAG is a lysosomal enzyme present in high concentrations in the proximal tubular cells: its large molecular weight does not permit glomerular filtration and consequently raised urinary concentrations indicate tubular dysfunction (Kunin et al, 1978; Bosomworth et al, 1999). In this study, significantly lower concentrations of this biomarker were noted on day 14 of treatment with TNS compared with IV tobramycin, even after correcting for urinary creatinine, confirming the potential for targeted aerosol delivery to be less immediately tubulotoxic. To my knowledge there has been only one case report of acute renal failure in CF in association with inhaled tobramycin therapy (Hoffmann et al, 2002). However, in this case the preceding prolonged use of ciprofloxacin (itself nephrotoxic) could have contributed to the renal insult with subsequent decreased renal drug elimination leading to an inappropriately high aminoglycoside level several hours after the last dose.

Although Steinkamp (Steinkamp et al, 1986) reported that inhaled tobramycin did not alter urinary NAG activity in four CF patients, the doses used were low (40 to 80mg bd) and negligible amounts were detected in the serum (<1.0 μg/ml in all

cases). Conversely, Ring (Ring et al, 1998) showed a correlation between urinary NAG and the cumulative dose of nebulised gentamicin in 20 CF subjects: whether gentamicin is more nephrotoxic than tobramycin is debatable. Only eight studies (Reed et al, 1981; Steinkamp et al, 1986; Godson et al, 1988; Hugli et al, 1992; Glass et al, 2005; Smyth et al, 2005; Etherington et al, 2007; Halacova et al, 2008) have measured urinary NAG excretion during IV aminoglycoside therapy in patients with CF, and different dosing regimens, laboratory assays and reporting methods make direct comparison difficult.

The effect on enzymuria by treatment with the IV colistin/TNS arm in this study was minimal. Although a previous report (Etherington et al, 2007) demonstrated a small rise in urinary NAG with IV colistin, that study combined colistin with a number of other IV antipseudomonal agents that have been linked with enzymuria *per se*. These observations need to be confirmed in larger controlled studies but if colistin were to exert a toxic effect on this part of the nephron, this may explain the enhancement of long term aminoglycoside-induced nephrotoxicity by colistin therapy, previously demonstrated in chapter 4. This subject merits further investigation.

In summary, new approaches to aminoglycoside therapy in acute CF exacerbations are needed. When this study was conducted, TNS (TOBI®) was the only high dose aminoglycoside licensed in the UK for nebulised use in CF patients, having been shown to be effective with a good safety profile in chronic intermittent (month on-month off) use (Burns et al, 1999; Ramsey et al, 1999; Moss, 2002; Gibson et al, 2003b). An alternative formulation (Bramitob®:tobramycin 300mg/4ml Nebuliser Solution, Chiesi Limited, Cheshire, UK) has since been licensed for long term aerosolised therapy in CF. My results suggest that the use of TNS (TOBI®) in acute CF exacerbations is also effective, safe and well tolerated with immediate renal sparing properties. Larger studies are needed to confirm this effect, explore how it

relates to long term nephrotoxicity with repeated exposure and whether the findings of this pilot study could be reproduced with Bramitob®.

8.5 TABLES

Table 8.5.1 - Changes in FVC, WCC and CRP with treatment

Test	*TNS*		*IV tobramycin*		*Mean difference between treatments*	*95% CI for difference*	*P value*
	Mean change	*SD*	*Mean change*	*SD*			
FVC % predicted	18.6	14.6	13.1	8.6	5.5	-12.9 to 1.9	0.16
WCC	-0.9	4.0	0.7	3.8	1.6	-0.8 to 4.1	0.19
CRP	-16.2	22.7	-20.3	32.4	4.2	-21.5 to 13.2	0.64

Table 8.5.2

Number (%) of patients reporting treatment emergent AE.

	TNS	*IV tobramycin*
aggrevated cough	4 (20)	7 (35)
increased sputum	4 (20)	4 (20)
haemoptysis	1 (5)	0 (0)
acute bronchospasm / chest tightness	0 (0)	0 (0)
hoarseness	6 (30)	2 (10)
pharyngitis	3 (15)	2 (10)
rhinorrhoea	5 (25)	3 (15)
conjunctival hyperaemia	1 (5)	0 (0)
tinnitus	0 (0)	2 (10)
dizziness	1 (5)	3 (15)
paraesthesia	1 (1)	1 (10)

8.6 FIGURES

Figure 8.6.1

Cross-over study design and data collection points

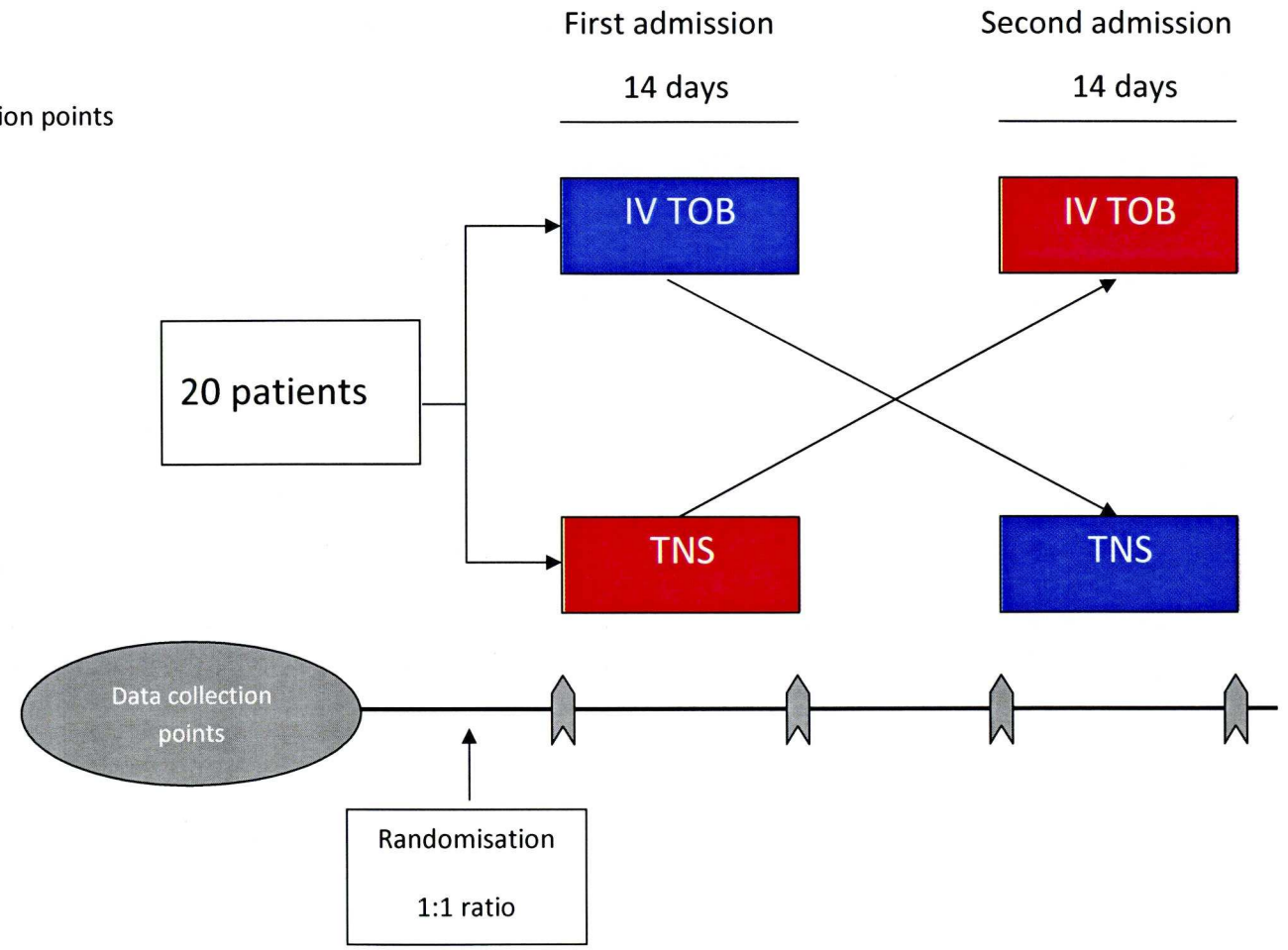


Figure 8.6.2

Changes in FEV1% predicted during the two treatment episodes.

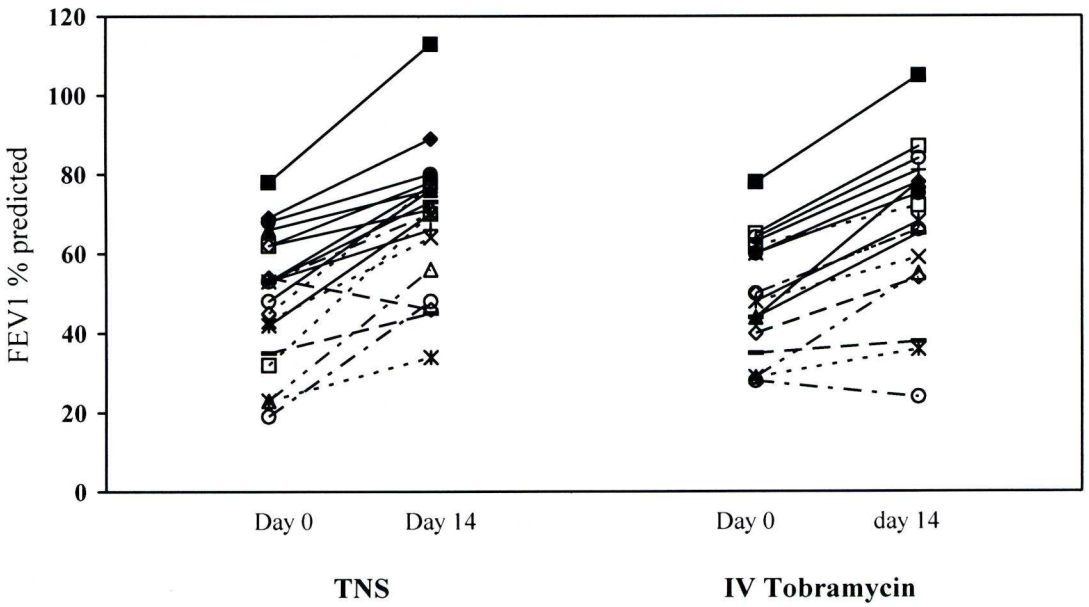


Figure 8.6.3

Effect of first treatment on the time to next exacerbation, P<0.001.

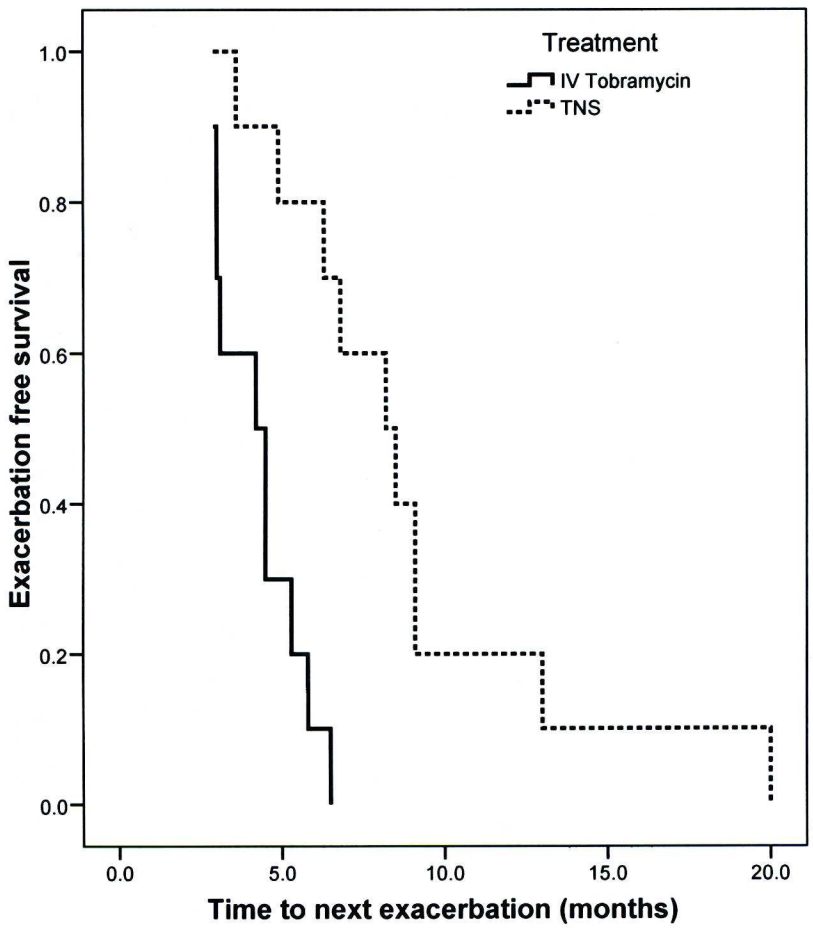
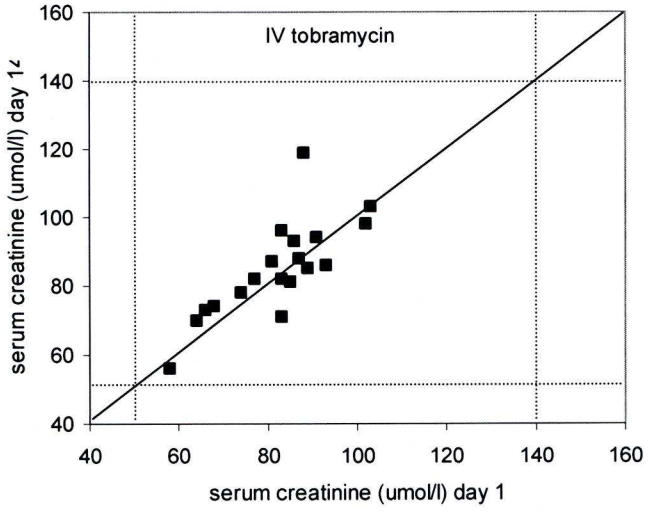


Figure 8.6.4

Changes in serum creatinine on treatment. Dotted lines represent local laboratory reference range.

(a) IV tobramycin
(b) TNS



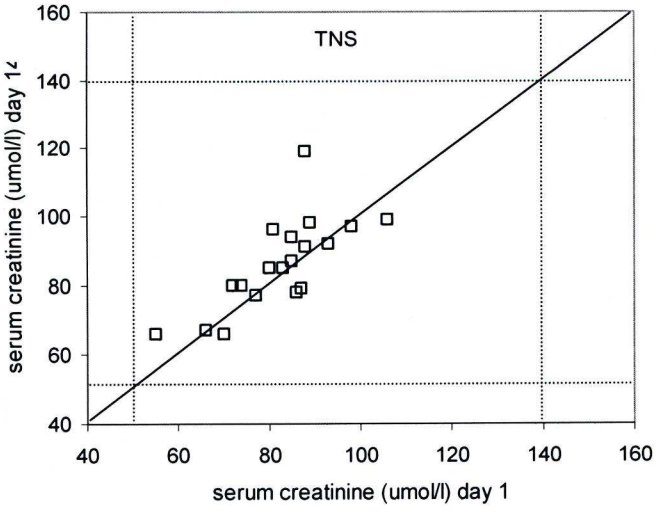
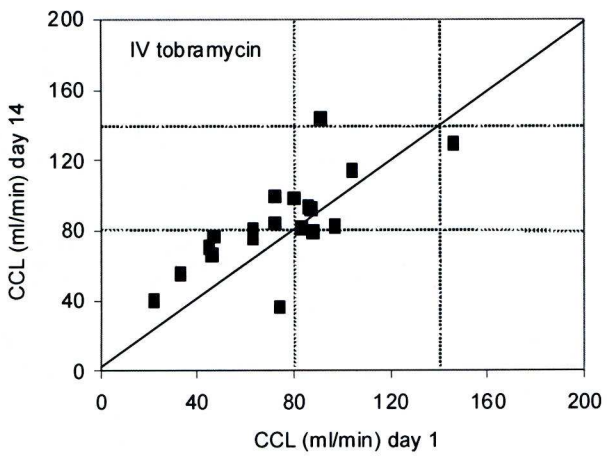


Figure 8.6.5

Changes in CCl on treatment. Dotted lines represent local laboratory reference range.

(a) IV tobramycin
(b) TNS



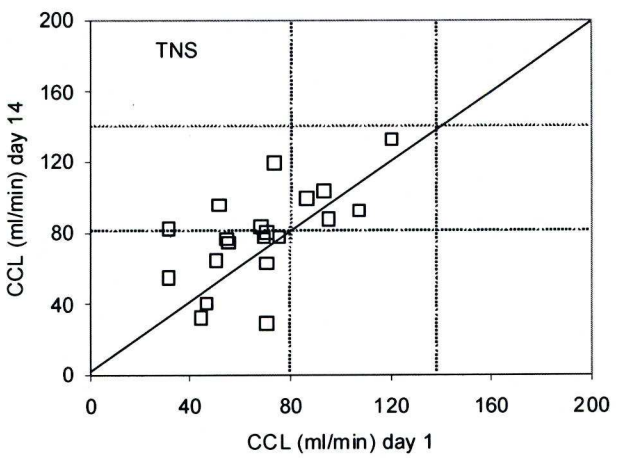
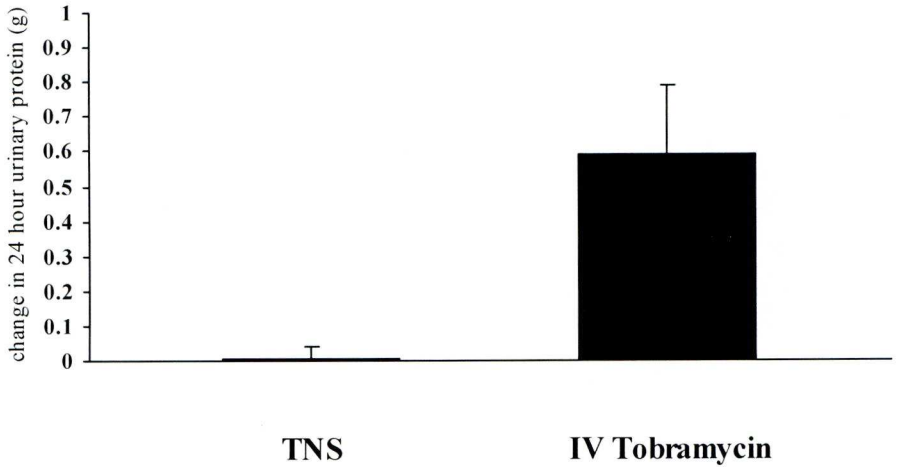


Figure 8.6.6

Changes in urinary protein leak (g/24hour) during the two treatment episodes (mean + standard error), P<0.001.



CHAPTER NINE

Does fosfomycin have a nephroprotective effect in CF?

Pilot cross over study of intravenous tobramycin and colomycin with and without fosfomycin in the treatment of pulmonary exacerbations in adult CF patients

9.1 INTRODUCTION

P. aeruginosa isolates from CF patients are becoming increasingly resistant to conventional antipseudomonal antibiotics (Pitt et al, 2003). The Liverpool epidemic strain (LES) of *P. aeruginosa*, now also isolated in most other UK CF centres (Scott and Pitt, 2003), is multi drug resistant, usually only predictably sensitive to tobramycin (TOB) and colistin (COL) *in vitro*. However, a previous study presented in chapter 4 confirmed a significant link between repeated intravenous (IV) use of these agents in the treatment of pulmonary exacerbations in CF and increasing nephrotoxicity. The continuing application of these antibiotics in CF, presently unavoidable, necessitates reduction of their dose limiting renal toxicity without compromising their concentration-dependent bactericidal effect.

Fosfomycin (1,2-epoxypropylphosphonic acid; FOM) was originally isolated from *Streptomyces fradiae* and other *Streptomyces* species (Hendlin et al, 1969) but is now produced synthetically. It is a unique broad spectrum bactericidal antibiotic chemically unrelated to any other known antimicrobial agent (Forsgren and Walder, 1983). FOM has useful activity against *P. aeruginosa* (Forsgren and Walder, 1983; Reeves, 1994), good lung tissue and biofilm penetration following IV injection (Farago et al, 1980), and evidence from experimental animal models suggests that it may attenuate the nephro- (Inouye et al, 1982; Fujita et al, 1983; Fujita, 1984; Morin et al, 1984) and oto-toxicity (Ohtani et al, 1984; Ohtani et al, 1985) of aminoglycosides when coadministered. Indeed its efficacy in combination IV therapy for the treatment of multiresistant *P. aeruginosa* CF exacerbations was reported recently (Mirakhur et al, 2003). However, its putative renoprotective properties have not been evaluated in this patient group. This chapter reports the results of a pilot prospective randomised cross over study of TOB+COL *vs* TOB+COL+FOM in the treatment of pulmonary exacerbations in a cohort of adult CF patients, paying particular attention to changes in renal function.

9.2 PATIENTS AND METHODS

9.2.1 Study population

I recruited 18 CF patients (mean [SD] age: 21.8 [3.4] years, forced expired volume in 1 second (FEV1): 59.3 [15.1] % predicted, body mass index (BMI): 21.2 [2.4] kg/m^2, 10 males), chronically infected with multiresistant morphotypes of LES *P. aeruginosa* who were admitted to the Liverpool adult CF unit with pulmonary exacerbations. Chronic infection was defined as 3 or more positive sputum cultures within the previous 12 months (Cystic Fibrosis Trust Control of Infection Group 2001), and multi-resistance as resistance to at least 2 of the 3 standard antipseudomonal antibiotic classes (Cystic Fibrosis Trust Antibiotic Group 2002). An exacerbation was defined as worsening respiratory symptoms accompanied by spirometric reduction (Rosenfeld et al, 2001a). Patients with known hypersensitivity to aminoglycosides, colistin or fosfomycin, significant haemoptysis or new radiographic changes, a history of *Burkholderia cepacia* complex isolation in the preceding 12 months, and those who had received any aminoglycoside (IV or nebulised) therapy during the previous 3 months or any additional antipseudomonal antibiotic or non-steroidal anti-inflammatory analgesic in the 2 weeks prior to randomisation were excluded. Four had CF-related diabetes at enrolment and no new cases of diabetes were diagnosed in the remainder over the study period. Changes to maintenance therapy were not permitted within 14 days of the exacerbations. None had fever or engaged in physical exercise for 48 hours prior to recruitment.

Patients gave written informed consent and the local ethics committee approved the study protocol.

9.2.2 Study design

Three antibiotics, all administered IV, were used in the study:

1. Tobramycin (80 mg / 2 ml, Mayne Pharma Plc, Warwickshire, UK) at a standard mean daily dose of 7.6 mg/kg [SD 0.8] with COL and 7.9 mg/kg [0.9] with COL+FOM in 2 – 3 divided doses [P=0.82]. According to local protocols, tobramycin levels were measured pre and one hour post the fourth administration and the dose adjusted to achieve a trough level of < 2.0 mg/l and a peak level of 6-10 mg/l. Subsequently, levels were measured as needed to ensure therapeutic serum concentrations.

2. Colistimethate sodium (Colomycin®, dry powder for injection, infusion or inhalation. Forest Laboratories Ltd, Kent, UK) at a fixed dose of 2 megaunits tid.

3. Fosfomycin disodium (5 g powder for reconstitution, Idis Pharma, Weybridge, UK) at a fixed dose of 5 g tid.

At the first exacerbation, patients were randomised in a 1:1 ration to receive 14 days of TOB+COL or TOB+COL+FOM. This is in line with the UK CF Trust guidelines advocating a minimum of 2 anti-pseudomonal antibiotics to treat pulmonary exacerbations (Cystic Fibrosis Trust Antibiotic Group 2002). When each patient was re-hospitalised at a later date with a second exacerbation, they received the alternative antibiotic combination at the same doses.

Assessment was performed on admission (day 1) and on day 14 and included clinical evaluation, spirometry, routine blood tests, 24 hour urine collections/spot urines and sputum sampling for culture and sensitivity.

9.2.3 Outcome measures

The primary efficacy end point of the study was the antibiotic-related change from baseline to day 14 in urinary levels of the proximal tubular enzymes N-acetyl-β-D-glucosaminidase (NAG) and alanine aminopeptidase (AAP). Renal indices also included:

- blood urea
- serum creatinine (SCr) and magnesium (Mg^{2+}) assays
- creatinine clearance (CCl) and total proteinuria/albuminuria measured from 24 hour urine collections
- urinary β_2-microglobulin (β_2M) levels

The two treatment strategies were also compared with regards to changes in % predicted FEV1 and forced vital capacity (FVC), peripheral blood white cell count (WCC) and C-reactive protein (CRP) as well as their impact on the time to next exacerbation. Safety endpoints included adverse events and serum tobramycin concentrations.

9.2.4 Laboratory methods

For measurements of serum tobramycin levels, serum and urine creatinine concentrations, calculation of mCCls, quantitation of 24-hour urinary protein elimination and urinary NAG, AAP and β_2M levels please refer to chapter 3 (General laboratory methods). The effects of varied urine concentration were minimised by expressing NAG, AAP and β_2M level as a ratio of urine creatinine. Results are reported as units/mmol creatinine (NAG and AAP) or mcg/mmol creatinine (β_2M).

9.2.5 Statistical analysis

Baseline demographics were compared using a paired t-test or Chi-squared test. Within group changes in pulmonary function, blood and urine tests were assessed using a paired t test (parametric data) or the Mann-Whitney U test (non parametric data). Treatment effect, 95% CI, and P values were analysed using a t-test or Wilcoxon rank sum test as appropriate. Comparison of time to next exacerbation was conducted with Kaplan Meier survival curves and a log rank test. Results are presented as means (standard deviation, SD), unless otherwise stated. $P<0.05$ was considered significant. Analysis was conducted with SPSS for windows version 15.0.

9.3 RESULTS

All 36 treatment episodes concluded satisfactorily. Table 9.5.1 shows baseline biometric data at the start of each treatment which did not differ between the two treatments.

The mean interval between admissions was 4.6 months [SD 2.9; range 1 – 11]. Allowing for age change, baseline patient characteristics were similar and the remainder of their therapy was otherwise identical in the 2 inpatient episodes (azithromycin: 6 patients, NSAIDS: 2, rhDNase: 10, IV aminophylline: 2, subcutaneous bricanyl: 2).

9.3.1 Changes in renal function

Prior to treatment, urine dipstick was negative in all patients and none had pathological proteinuria. All had normal renal ultrasound scans at the preceding annual review and none had received organ transplantation or was being treated with cyclosporine. None had previous episodes of acute renal failure.

Urinary levels of the proximal tubular enzymes NAG and AAP after 14 days treatment with TOB+COL+FOM were significantly lower than those recorded after treatment with TOB+COL alone (table 9.5.2). The addition of FOM to TOB+COL also attenuated antibiotic-induced total urinary protein leak in a 24 hour collection but urinary albumin did not alter from baseline and did not differ between the two treatments. Although β_2M levels on the triple combination were numerically lower, the difference observed was not statistically significant. Except for comparable improvements in CCl, none of the other renal indices changed significantly with either treatment.

9.3.2 Recovery from exacerbation

The mean 14 day improvements in all surrogate markers of exacerbation resolution were similar for both treatments (table 9.5.3).

9.3.3 Sputum microbiology

Thirty one out of a possible 36 sputa were collected prior to treatment: all harboured multiresistant *P. aeruginosa* isolates that were only susceptible to tobramycin and colistin *in vitro*. Twenty eight isolates appeared resistant to FOM on standard disc diffusion tests.

P. aeruginosa eradication was not observed (29/31 remained positive, 2/31 did not submit samples at the end of treatment, 2 of the remaining 5 were able to produce sputum at the end of treatment, both were *P. aeruginosa* positive). Treatment-emergent superinfection with new pathogens was not recorded in any of these episodes.

9.3.4 Time to next exacerbation

The addition of FOM did not impact on the time to next exacerbation requiring hospitalisation. The interval between 2 consecutive hospital admissions was 4.2 (SD 2.9) and 5.0 (SD 3.2) months for patients treated initially with TOB+COL and TOB+COL+FOM respectively; χ^2 test 0.89; HR 1.52, 95% CI 0.59-4.41, P=0.35 (figure 9.6.1).

9.3.5 Safety

9.3.5.1 Adverse events (AEs)

There were no AEs warranting withdrawal of therapy. The incidence of AEs was comparable during both treatments: treatment-emergent AEs were recorded in 8 (44%) and 9 (50%) of TOB+COL and TOB+COL+FOM episodes respectively (table 9.5.4).

9.3.5.2 Serum tobramycin levels

Serum tobramycin assays were similar throughout both treatments and no toxic drug levels were recorded. Paired tobramycin assays (median 2, range 1-4 per patient per treatment) showed mean trough and peak levels of 1.1 (0.3) and 6.7 (1.6) *vs* 1.1 (0.5) and 6.6 (1.8) mg/l for TOB+COL and TOB+COL+FOM respectively.

9.4 DISCUSSION

Over the past 40 years, attention has been directed towards the evaluation of urinary enzymes as non-invasive biomarkers of renal tubular damage. They proved to be sensitive tools useful in the early diagnosis of acute renal injury before conventional laboratory assays become deranged (Trollfors et al, 1980; Sethi and Diamond, 1981; Mondorf, 1982). An elevation in urinary enzyme activity also helps

indicate the site of primary tubular damage because of their localisation in brush border (AAP) and tubular lysosomes (NAG) (Mondorf, 1982). Such tests have been applied in studies of nephrotoxicity caused by different agents including immunosuppressive drugs (Diener et al, 1981), contrast media (Schiavina et al, 1984; Tschakert et al, 1995), antibiotics (Gibey et al, 1981; Davey et al, 1983; Mondorf et al, 1983; Rybak et al, 1987), lead (dos Santos et al, 1994) and cadmium (Gatta et al, 1989; Mueller et al, 1989) exposure. Enzymuria and proteinuria have also been used to investigate preclinical renal impairment in conditions known to cause overt kidney disease including diabetes (Hong and Chia, 1998; Kalansooriya et al, 2007) and glomerulonephritis (Hultberg and Ravnskov, 1981; Holdt-Lehmann et al, 2000). Whether enzymuria predicts impending and/or chronic renal impairment remains to be clarified. Furthermore, no consensus has been reached as to which diagnostic indicator has the most accurate prognostic value.

Using these assays, I was able to demonstrate that incorporating FOM in the antibiotic treatment of CF pulmonary exacerbations afforded some protection against the immediate proximal tubular injury caused by the TOB+COL combination. Previously only described in animal models (Inouye et al, 1982; Fujita et al, 1983; Fujita, 1984; Morin et al, 1984), this is the first report to reproduce the nephroprotective properties of FOM against aminoglycoside (and possibly colistin)-induced tubulotoxicity in CF patients. Prior studies of FOM and the human kidney had been largely confined to its ability to attenuate the renal insult of chemotherapy, particularly cisplatin-based regimens (Umeki et al, 1988; Hayashi et al, 1997; Rojanasthien et al, 2001).

FOM is taken up actively into bacterial cells through two nutrient transport systems present in various bacteria (including *P. aeruginosa*) and inhibits the initial step in cell wall synthesis (Kahan et al, 1974; Reeves, 1994). However, *in vitro* susceptibility testing for FOM against *P. aeruginosa* requires the presence of glucose-6-

phosphate, which is not routinely incorporated into standard sensitivity testing agars. Thus without this, sensitive strains may appear resistant (Forsgren and Walder, 1983). Indeed, most of the *P. aeruginosa* isolates in our cohort were reported to be FOM resistant. Lack of reliable and easily performed methods of evaluating *in vitro* activity of FOM against *P. aeruginosa* is an important issue that hampers the clinical assessment of this antibiotic. So far, the only approved susceptibility testing method for fosfomycin is agar dilution (Clinical and Laboratory Standards Institute 2005a) which is both laborious and time consuming. Studies examining alternative susceptibility tests concluded that the minimum inhibitory concentration (MIC) data for FOM against *P. aeruginosa* is markedly dependent on the method used and none of broth microdilution, disc diffusion or E-test strips are reliable alternatives to agar dilution (de Cueto et al, 2006; Lopez-Cerero et al, 2007). Resolution of an exacerbation does not require *P. aeruginosa* eradication but is usually associated with a reduction in sputum *P. aeruginosa* density (Smith et al, 1988; Regelmann et al, 1990). I did not quantify sputum bacterial load before or after antibiotic treatment. However, FOM did not prolong time to the next exacerbation suggesting it did not potentiate the effect of TOB+COL on Pseudomonal colony counts. Admittedly, this is somewhat speculative but there appears to be a dissociation between its antimicrobial and nephroprotective effects.

Despite this, FOM may be useful when co-administered with other antibiotics for a number of reasons. The vigorous inflammatory response in the CF lung encourages *P. aeruginosa* to form microcolonies surrounded by negatively charged polysaccharides (the biofilm or glycocalyx). This biofilm allows the persistence of the organism in the face of specific antibodies and antibiotics (Hoyle and Costerton, 1991; Anwar et al, 1992). However, *in vitro* FOM does not react with the negatively charged glycocalyx and *in vivo* therefore may be better poised to penetrate the biofilm (Kumon et al, 1994). A synergistic *in vitro* effect has been demonstrated in combination with ofloxacin against *P. aeruginosa* growing in a biofilm (Kumon et al, 1995) and with ciprofloxacin against *P. aeruginosa* isolates from CF patients

(Figueredo and Neu, 1988). This may be because FOM acts on different synthetic pathways, demonstrating synergy against *P. aeruginosa* when used in combination with a wide variety of other antibiotics including β-lactams, aminoglycosides, macrolides and tetracyclines (Daza et al, 1977; Perea et al, 1977; Ullmann and Lindemann, 1980; Takahashi and Kanno, 1984; Courcol and Martin, 1987; Tessier and Quentin, 1997; Schulin, 2002).

Because FOM acts on synthetic pathways unaffected by other agents, the potential for the development of cross resistance with other classes of antibiotics is reduced (Perea et al, 1977; Woodruff et al, 1977). Furthermore, FOM has an excellent side effects profile. Whilst the oral (calcium salt) preparations can cause gastrointestinal side effects in 2-8% of cases (Jardin, 1990; MacGowan et al, 1990; Mayama et al, 1993), there are no reports in the literature of significant side effects with the IV (sodium salt) formulation. Its potential to ameliorate aminoglycoside (and perhaps colistin)-associated nephrotoxicity demonstrated in this study adds further support to its utility in the CF population.

I observed similar peak and trough serum tobramycin levels on both treatments, which rules out improved aminoglycoside elimination as the underlying mechanism for the apparent nephroprotection.

The primary toxic effect of aminoglycosides is on the lysosomal system within the proximal tubule (Mingeot-Leclercq and Tulkens, 1999). NAG is a lysosomal enzyme present in high concentrations in the proximal tubular cells. Furthermore, its molecular weight (140KD) does not permit glomerular filtration. Thus, raised urinary NAG levels have been shown to reflect proximal renal tubular dysfunction (Bosomworth et al, 1999). Eight studies have previously described elevated urinary NAG excretion during IV aminoglycoside therapy in patients with CF (Reed et al,

1981; Steinkamp et al, 1986; Godson et al, 1988; Hugli et al, 1992; Glass et al, 2005; Smyth et al, 2005; Etherington et al, 2007; Halacova et al, 2008). In addition, Etherington et al (Etherington et al, 2007) demonstrated a much smaller and transient rise in NAG levels after 2 week treatment with colistin at doses recommended by the CF Trust guidelines and similar to those employed in my study. This may explain the observation that colistin did not impair GFR with long term use in a large cross sectional study of CF adults although it appeared to enhance the nephrotoxicity of tobramycin when co-administered (Chapter 4). Thus, the mechanisms of tubular toxicity for both drugs have been postulated as similar. Consistent with these reports, the TOB+COL combination in my study generated a high NAG signal. The addition of FOM attenuated this signal, suggesting that FOM helps preserve lysosomal membrane integrity, as previously suggested in rat models (Morin et al, 1978; Inouye et al, 1982; Fujita et al, 1983; Fujita, 1984; Morin et al, 1984). A similar mechanism may account for protection against aminoglycoside-related ototoxicity.

I have found no published communications regarding urinary AAP application in CF. Several investigators have reported significant correlations between AAP excretion and decreased renal function in other patient groups (Davey et al, 1983; Rybak et al, 1987; Holdt-Lehmann et al, 2000; Kalansooriya et al, 2007). As with other indicators of early renal injury, elevations of AAP in urine precede increases in serum creatinine in patients with renal toxicity (Diener et al, 1981; Gibey et al, 1981; Davey et al, 1983). The AAP assay, as described by Jung and Scholz (Jung and Scholz, 1980), has been consistently sensitive enough to detect differences in enzymuria between various treatment regimens (Rybak et al, 1987; Nix et al, 1997) and different severity groups of nondrug-related renal disease (Holdt-Lehmann et al, 2000). I chose to use AAP as a marker in this study because of its specificity to the proximal tubule cell, since this is the area in which aminoglycosides inflict their damage. Accordingly, observed changes in urinary AAP mirrored those of NAG.

β_2M was used for comparison purposes since it has traditionally been used in clinical practice to detect renal tubular damage (Wibell, 1978; Stosic et al, 1989; Viergever and Swaak, 1989; Ikeda et al, 2009). It is more of a functional assay: NAG and AAP levels reflect tubular structural integrity whereas β_2M acts as a surrogate marker of functional competency of the proximal tubular epithelium (Viau et al, 1986). β_2M is a low molecular weight (12 KD) plasma protein synthesised by the nucleated cells of the body and is normally filtered at the glomerulus and completely reabsorbed by the proximal tubules where it is catabolised (Wibell, 1978). Interference with proximal tubular reabsorption, due to a variety of tubulointerstitial diseases (Diadyk et al, 1991) and tubulotoxins (Tyner, 1999; Kinai and Hanabusa, 2005), will cause enhanced excretion of β_2M and other low molecular weight proteins such as alpha-1-microglobulin and retinol-binding protein into the urine (Bernard et al, 1982). Indeed, increased urinary β_2M leak has been found to correlate with altered renal function secondary to aminoglycoside exposure (Trollfors et al, 1980; Gibey et al, 1981; Rybak et al, 1987).

Both AAP and β_2M levels rose significantly after TOB+COL therapy. Any contribution from colistin is at present unconfirmed. FOM reduced AAP levels significantly. We could therefore hypothesise that it also preserves the apical brush border cell membrane in the tubular epithelium in a similar mechanism to that in the lysosome. In comparison, the impact of FOM on β_2M levels was less dramatic. Large inter-patient variability in the present study may have accounted for this apparent discrepancy between NAG/AAP *vs* β_2M excretion. Unlike shorter drug challenges in animal studies (Inouye et al, 1982; Fujita et al, 1983; Fujita, 1984; Morin et al, 1984), continued exposure to TOB/COL over two weeks may have meant that despite the structural preservation, functional deterioration would ultimately become evident, albeit to a lesser extent. In addition, at urine pH<5.5, β_2M degrades very rapidly at 37°C and it is likely that this degradation can be initiated in the bladder (Donaldson et al, 1989; Blumsohn et al, 1991). I therefore did not use morning voids (which tend to be more acidic) and adjusted all samples to a pH of 7 immediately after

collection but it is possible this does not entirely eliminate a confounding effect. Nevertheless, there appeared to be a trend towards lower urinary β_2M when FOM was added to TOB+COL. In the absence of disease increasing β_2M production (eg. lymphoma), a rise in GFR reduces serum β_2M levels, whilst saturating proximal tubular reabsorptive capacity resulting in higher urine levels (Wibell, 1978). However CCl improved to the same extent in both arms of the study, so this can not explain the lower β_2M signal with FOM. Finally, in proteinuric states, an increased urinary excretion of β_2M may not simply reflect tubular damage but could also result from decreased tubular reabsorption due to competitive mechanisms (Branten and Wetzels, 1999). Although total protein leak was higher in TOB+COL, albuminuria levels (indicator of glomerular proteinuria) were similar suggesting that protein load arriving at the proximal tubule was not significantly different in the two treatment arms. Furthermore, none had fever or engaged in physical exercise for 48 hours prior to recruitment as both would physiologically accentuate proteinuria.

Current monitoring of renal injury (blood urea nitrogen [BUN] and serum creatinine) is not sufficiently sensitive to detect early changes in renal tubular function. Toxicity is not detected until serious functional damage is evident with a large reduction in nephron mass. Hence, despite enzymuria and proteinuria, traditional indices such as urea, creatinine and Mg^{2+} did not change. Consistent with earlier reports and data presented in chapters 7 and 8, CCl improved after reversal of the volume deplete hypercatabolic state characteristic of the CF exacerbation.

Patients with CF who are chronically infected with *P. aeruginosa* will require repeated courses of IV antipseudomonal antibiotics for pulmonary exacerbation. This frequent use of a limited selection of antibiotics encourages the development of resistance and many adult CF patients now harbour multiresistant *P. aeruginosa* strains. In addition, repeated use of the same antibiotics results in patient intolerance/sensitisation and increased toxicity, including cumulative renal

impairment. FOM is a unique bactericidal antibiotic which can help expand the range of antipseudomonal therapy available for CF patients and, in my experience, FOM appeared safe and well tolerated with valuable immediate renal sparing effects. Whether this translates into clinically meaningful preservation of renal function needs to be assessed in larger long-term studies.

9.5 TABLES

Table 9.5.1 - Baseline biometric data at the start of each treatment

	TOB+COL (N=18)		TOB+COL+FOM (N=18)	
	Mean	SD	Mean	SD
FEV1 % predicted	48.3	13.1	47.2	13.8
FVC % predicted	67.9	10.0	65.4	8.1
BMI (kg/m^2)	21.9	2.6	21.6	2.7
Blood urea (mmol/l)	4.4	0.9	4.2	0.8
Serum creatinine (µmol/l)	81.6	10.1	82.0	11.8
Serum Mg^{2+} (mmol/l)	0.90	0.15	0.83	0.17
CCl (ml/min)	84.3	21.2	81.4	19.7

Continues on the following page

Table 9.5.1 continued

	TOB+COL (N=18)		TOB+COL+FOM (N=18)	
	Mean	SD	Mean	SD
24 hr urine protein excretion (g)	0.08	0.05	0.07	0.05
Urinary albumin (mg/mmol)	1.6	0.7	1.8	0.6
Urinary NAG (iu/mmol)	0.46	0.71	0.50	0.57
Urinary AAP (iu/mmol)	0.95	0.52	0.84	0.54
Urinary β_2M (mcg/mmol)	23	13	20	9
TOB daily dose (mg/kg)	7.6	0.8	7.9	0.9
COL daily dose (megaunits)	6	-	6	-
FOM daily dose (g)	N/A	-	15	-

Table 9.5.2 - Changes in renal function indices

Test	TOB+COL		TOB+COL+FOM		Mean difference between treatments	95% CI for difference	P value
	Mean change	SD	Mean change	SD			
Urea	0.68	1.64	0.62	1.59	-0.06	-1.154 to 1.034	0.912
Creatinine	4.33	10.22	2.17	9.78	-2.16	-8.936 to 4.616	0.521
CCl	17.28	17.13	19.89	14.64	2.61	-8.128 to 13.348	0.635
Mg^{2+}	-0.05	0.02	0.05	0.19	0.1	-0.032 to 0.232	0.13

Continues on the following page

Table 9.5.2 Continued

Test	TOB+COL		TOB+COL+FOM		Mean difference between treatments	95% CI for difference	P value
	Mean change	SD	Mean change	SD			
Total proteinuria	0.27	0.20	0.04	0.07	-0.23	-0.331 to -0.129	0.0001
Albuminuria	0.19	0.47	0.25	0.60	0.06	-0.313 to 0.417	0.7732
NAG	2.74	2.83	0.86	0.97	-1.88	-3.313 to -0.447	0.0117
AAP	30.1	33.4	11.8	11.04	-18.3	-35.15 to -1.45	0.0342
β_2M	147	180	63	91	-84	-180.6 to 12.66	0.0862

Table 9.5.3 - Changes in FEV1, FVC, WCC and CRP with treatment

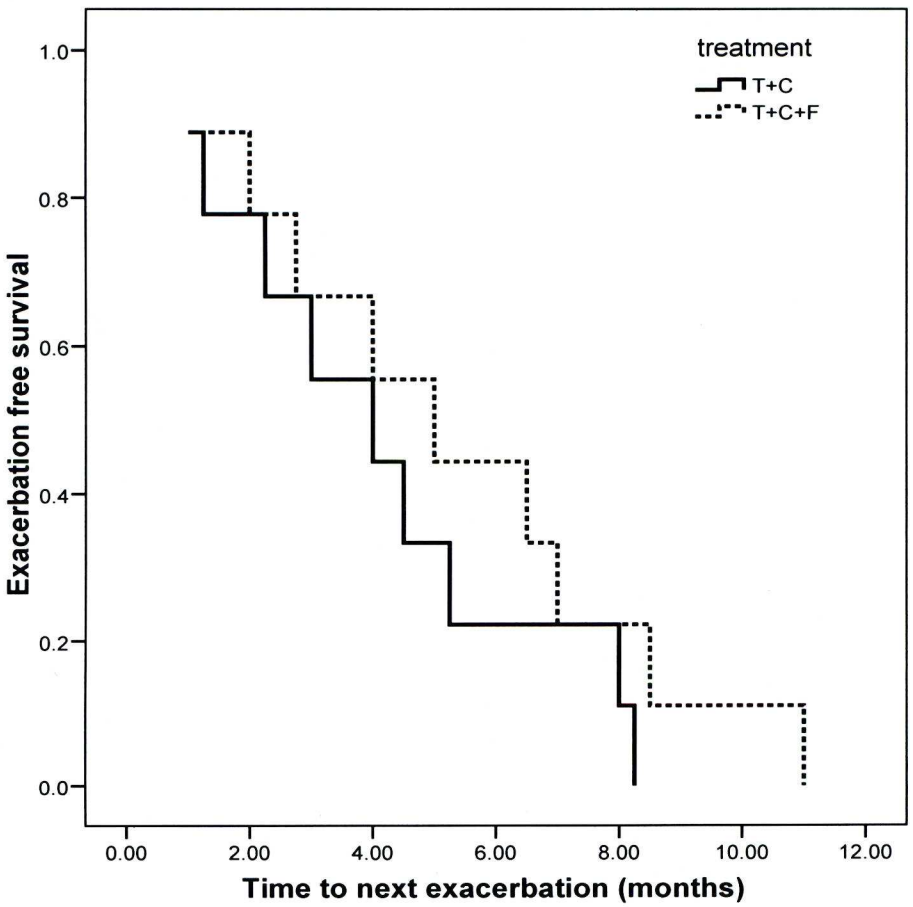
Test	TOB+COL		TOB+COL+FOM		Mean difference between treatments	95% CI for difference	P value
	Mean change	SD	Mean change	SD			
FEV1 % predicted	+12.6	8.0	+11.8	5.9	-0.8	-5.6 to 4.0	0.73
FVC % predicted	+14.1	7.7	+15.9	6.9	1.8	-3.15 to 6.75	0.46
WCC	-2.9	6.3	-3.3	4.1	-0.4	-4.0 to 3.2	0.82
CRP	-27	26.8	-34	30.0	-7	-26.3 to 12.3	0.47

Table 9.5.4 - Number (%) of patients reporting treatment-emergent AE

AE	TOB+COL	TOB+COL+FOM
aggrevated cough	3(17)	3(17)
increased sputum	3(17)	4(22)
acute bronchospasm / chest tightness	2(11)	0(0)
Sore throat/hoarseness	2(11)	2(11)
reduced hearing	2(11)	1(11)
tinnitus	2(11)	0(0)
dizziness	3(17)	1(17)
Paraesthesia	1(6)	1(6)
Nausea	2(11)	3(17)
diarrhoea	1(6)	0(0)
Treatment episodes with any reported AE	8(44%)	9(50%)

9.6 FIGURES

Figure 9.6.1 - Time to next exacerbation requiring hospitalisation per treatment group



CHAPTER TEN

Concluding remarks

10.1 Renal impairment in cystic fibrosis: Is there cause for concern?

Although suppurative lung disease is responsible for most of the morbidity and mortality in CF, expression of the CFTR gene mutation in many other organs makes it a truly multi-system disease (Boyle, 2007). Life expectancy in patients with CF has increased over the years. The UK CF data estimates life expectancy is now greater than 50 years for those born in 2000 (Dodge et al, 2007). Complications associated with the extended longevity of patients with CF include infection of the lung with multi-resistant pathogens, diabetes, osteoporosis, gastric reflux and liver disease. Treatment of these resistant pathogens introduces new challenges: they dictate repeated use of a handful of powerful, often toxic antimicrobials (Boyle, 2007). In addition, a long history of cystic fibrosis-related diabetes increases the prevalence of its microangiopathic complications (Brennan et al, 2004). As a result, the focus has turned recently toward potential, if not actual, renal complications as highlighted by the present study. Chapters 4 and 5 demonstrate that both acute renal failure (now termed acute kidney injury, AKI) and long term reduction in renal reserve are a genuine concern in these patients and there is a need for better tools to monitor renal function and innovative treatment strategies aimed at nephroprotection.

10.2 Renal function in CF: what is already known? what did this study add? and what is new since?

Renal expression of the CFTR gene mutation has been well documented, yet its exact role is uncertain. Its contribution to the risk of patients with CF developing renal disease is unknown. However, the contribution of long-term exposure to nephrotoxic aminoglycoside antibiotics and renally toxic immunosuppressants in CF lung transplant recipients is well recognised (Stephens and Rigden, 2002), though the prevalence and magnitude of this toxicity remained largely unmeasured.

Recently, a survey was conducted to document current Australian practices of administration and monitoring of aminoglycosides in patients with CF (Soulsby et al,

2009). Eighteen out of the 28 CF units surveyed reported having identified renal toxicity in their CF patient population that they associated with aminoglycoside use. There was no consensus as to the best method of monitoring renal function in CF, so the real extent of the problem is not yet fully understood. The prospective cross sectional study presented in chapter 4 suggests significant renal dysfunction with repetitive aminoglycoside use in adults with CF is likely. In this study between 31% and 42% of patients had a measured creatinine clearance that was below the normal range in our laboratory. My data indicate that lifelong treatment with nephrotoxic agents (and/or disease progression in adults with CF – though it is not possible to separate the two effects) has the potential to impact on renal function as patients age. Whilst previous publications did not include polymyxins, I acknowledge that the concomitant use of IV colistin (also a potential nephrotoxin) may have increased the prevalence of renal impairment seen in our patient population.

When the initial screening cross sectional study was conducted, the local laboratory lower limit of normal measured CCl (80 mls/min/1.73 m^2) was used. Forty two percent had reduced mCCl, averaging 62 ml/min/1.73 m^2. More recent UK CKD guidelines defined the thresholds of chronic kidney disease according to GFR, where values of 60-89 ml/min/1.73 m^2 represent a slight decrease in GFR and lower values are consistent with moderate to severe renal impairment (National Institute for Health and Clinical Excellence, September 2008). Several patients in the study presented in chapter 4 had only mild reductions in GFR and some may question the relevance of this. It is noteworthy however, that I encountered a relatively high number of episodes of ARF/AKI, some of these documented in patients who previously had normal or only slightly reduced GFR. There have been many case reports of aminoglycoside induced AKI in the CF literature (Samaniego-Picota and Whelton, 1996; Drew et al, 2002; Drew et al, 2003; Kennedy et al, 2005). These patients all received gentamicin as their aminoglycoside antibiotic. In chapter 5, I reported 8 cases of AKI in adults with CF where all patients received tobramycin three times a day; in some of these, creatinine clearance remained abnormal

several months after apparent resolution of the AKI episode. AKI may occur in all age groups. In 2007, Bertenshaw *et al* (Bertenshaw et al, 2007) surveyed the incidence of aminoglycoside-induced AKI in patients with CF in the UK between 1997 and 2004. They found that 15 of the 21 patients identified in the study had received gentamicin, 5 received tobramycin, and 1 patient had received both aminoglycosides. The youngest patient was only 4 months old and 14 patients were under 10 years of age. Renal function, defined crudely by serum creatinine levels, returned to normal in all these patients on cessation of the aminoglycoside. They concluded that AKI is increasingly recognized in patients with CF. They also suggested there may be other factors that have an impact on renal function in the adult CF population, for example, certain CF phenotypes may be predisposed to renal dysfunction, and cystic fibrosis-related diabetes. In the case controlled study subsequently conducted by the same group (Smyth et al, 2008) 24 cases of AKI were confirmed between 1997 and 2004 in the UK. This case series included the cohort I described in chapter 5. Of these 24 patients, 21 had received an aminoglycoside antibiotic within a week of their episode of AKI. This study demonstrated more conclusively that the use of aminoglycosides in patients with CF is a risk factor for AKI.

It is important to note that as life expectancy increases more patients with CF receive lung transplants. All patients receiving solid organ transplants are at risk of renal toxicity primarily due to the immunosuppressant therapy used (Ishani et al, 2002). Ishani *et al* reported renal function decline in over 90% of lung transplant recipients, especially in the first 6 months after their transplant, with up to 7% of patients ultimately developing end stage renal disease (ESRD). The signal is inconsistent however, with some reports indicating patients with CF did not appear to be at greater risk of this occurring than non-CF transplant recipients and others highlighting reduced renal survival post lung transplantations in CF compared with other patient groups (Ishani et al, 2002; Canales et al, 2006). The major risk factors for developing ESRD in this population are: lower GFR pre-transplant; a reduced

GFR measured 1 month post-transplant; the use of cyclosporin alone in the first 6 months and female gender (Broekroelofs et al, 2000).

10.3 How is renal function being monitored in the CF population at present?

In order to investigate the potential renal sparing properties of new antibiotics/antibiotic policies, the best method to measure renal function and monitor treatment induced changes has to be defined. Tan et al published a literature review to establish guidelines for the monitoring of renal toxicity in patients with CF receiving aminoglycosides, as well as best practice in relation to dose and administration (Tan et al, 2002b; Tan et al, 2003; 2005b). They concluded that "Adverse effects of treatment in patients with CF will become more common unless surveillance of aminoglycoside toxicity is performed" and recommended a series of tests that should be carried out during IV aminoglycoside therapy. These included therapeutic drug monitoring and measurement of serum magnesium, potassium, calcium, creatinine, and urea. Tan et al (Tan et al, 2003) subsequently carried out a survey of CF centres in the UK published in 2003. They reported that 78% of centres were monitoring for renal toxicity but only using creatinine and electrolytes. In a 2007 survey of 28 CF units in Australia, 96% also used plasma creatinine as the sole method of monitoring renal function. The frequencies of monitoring varied from daily to alternate weekly (Soulsby et al, 2009).

However, none of my 80 patients had elevated SCr outside the local reference range, despite subnormal *measured* CCl in up to 40%. Interestingly, Pedersen *et al* made a similar observation as early as 1987 (Pedersen et al, 1987). The authors raised concerns regarding cumulative damage caused by exposure to multiple courses of IV tobramycin. Their study identified that 40% of their patient population (n=46) had an abnormal *calculated* creatinine clearance but none had an elevated serum creatinine outside the normal range. However, they did not observe any clinically obvious chronic nephrotoxicity. Similarly, acute kidney tubular injury noted in the two cross-over studies presented in chapters 8 and 9 was not accompanied by significant changes in SCr.

In conclusion, a normal SCr in a CF patient is not synonymous with normal CCl nor normal tubular function.

GFR is the most commonly accepted parameter that accurately reflects renal function. An ideal endogenous marker for measuring GFR should be produced at a constant rate, should be eliminated only by glomerular filtration (with no subsequent tubular handling) and drugs should not affect the assay. Currently, GFR is estimated by using creatinine clearance determined in one of two ways:

1. Twenty-four hour urine collection
2. By using equations based on plasma creatinine

or using isotopic or non-isotopic clearance rates (the "gold-standard test"). All of these methods have drawbacks as discussed in chapter 6.

In this study, repeated urine collections from each patient were subjected to rigorous validation rules as originally applied by Cockcroft and Gault and subsequent authors (Cockcroft and Gault, 1976; Thakur et al, 1997) – (Chapters 4 and 6). However, measurement of GFR did not reflect acute tubular structural and functional changes induced by a course of antibiotics. The data presented in chapters 7, 8 and 9 indicate that, if anything, GFR improves in the majority of patients after a standard 14 day course of IV therapy. Improvement in the inflammatory hypercatabolic state and better hydration is likely to be responsible for this.

Instead, Tan *et al* (Tan et al, 2003) recommended the use of the CGF equation for estimating CCl in CF but this was not validated. One potential flaw with equation-derived estimation of CCl is that patients with CF often have low muscle mass (Miller et al, 1982) leading to a lower than normal production of creatinine and thus low SCr levels. This can lead to over-estimation of creatinine clearance and an underestimation of their loss of renal function. In chapter 6, I attempted to validate several equations commonly used for estimating CCl including both the CGF and the

aMDRD equations. I compared 10 equations with stringently validated 24-hour urine collections as the control. None of these equations could be used reliably to assess renal function in patients with CF, especially as they tended to grossly overestimate CCl, particularly in the subgroup with diminished GFR, limiting their utility as a screening tool for renal impairment in the outpatient clinic.

It is important that there is an increased awareness of these limitations and the need for a simple, but accurate test to be available for monitoring renal function in CF which is capable of identifying renal impairment earlier rather than later (Tan et al, 2002b; Glass et al, 2005; Chapter 6).

10.4 Alternative methods for measuring and monitoring renal function in CF

There is interest in finding less invasive and time consuming ways to measure and monitor renal function compared to the gold-standard clearance tests. These include quantification of urinary enzymes and proteins as described in chapters 7, 8 and 9. Since tubular dysregulation is an early event in the pathogenesis of aminoglycoside nephrotoxicity, it seems reasonable that strategies directed at assessing tubular structural integrity and function would yield important functional and prognostic data. The measurement of increased levels of small proteins in urine such as NAG, AAP, β2M, alpha 1 microglobulin, retinol binding protein, and alpha/pi S-glutathione transferases is associated with tubular cell dysfunction. Therefore their straightforward measurement could provide a powerful tool in patient monitoring and ongoing clinical assessment of renal function.

Aminoglycosides affect renal function by causing toxicity primarily in the lysosomal system within proximal tubular cells. NAG is a lysosmal enzyme found in high concentrations in the renal proximal tubular cells and is highly sensitive to acute renal insults. Thus urinary NAG is a marker for tubular damage but does *not* measure renal function *per se*; rather it demonstrates acute injury in the presence of a variety of tubulotoxins and disease processes involving the kidneys. Eight studies have measured urinary NAG as an indicator of acute drug-induced

nephrotoxicity in CF (Reed et al, 1981; Steinkamp et al, 1986; Godson et al, 1988; Hugli et al, 1992; Glass et al, 2005; Smyth et al, 2005; Etherington et al, 2007; Halacova et al, 2008): a consistent observation has been that urinary NAG increases in patients receiving IV aminoglycosides but the changes are reversible and return to baseline at follow up, although the reported follow up interval was variable. In the TOPIC study (Smyth et al, 2005) which assessed the safety and efficacy of once versus three times daily IV tobramycin, changes in urinary NAG were compared between the two groups. Urinary NAG increased in all 107 patients who completed the study with the increase in NAG levels being 33% lower in those receiving once daily dosing. Interestingly, in the TOPIC study the changes in NAG levels were only significant in children and not in the adults although the relevance of this was not investigated. Using NAG and other renal biomarkers I was able to:

1. Follow longitudinally the time scale of tubular recovery after a standard 14 day IV tobramycin challenge and was able to demonstrate that a high antibiotic pressure with shorter drug exposure-free periods lead to incremental tubular injury, recovery of which may be slower with consecutive treatments (chapter 7).

2. Demonstrate potential renal sparing effects from the use of aerosol delivery of tobramycin in the treatment of acute pulmonary exacerbations without compromising treatment efficacy (chapter 8). Similar benefits were shown in the cross-over study adding fosfomycin to a combination of IV tobramycin and colistin (chapter 9).

This invites a review of our current antibiotic prescribing practices and emphasises the need for fluent communication between CF units and their local or regional microbiology laboratories to help interpret sputum culture results and expand antibiotic choice. A ceftazidime-resistant transmissible strain of *P. aeruginosa* (the

Liverpool Epidemic Strain [LES]) was first described by the neighbouring paediatric CF unit in 1996 (Cheng et al, 1996). This strain rapidly spread in our unit (and other CF units in the UK (Scott and Pitt, 2004)), causing chronic infection in nearly 80% of the Liverpool adult CF population. Reports of excess morbidity and treatment burden from transmissible strains (Jones et al, 2002; Al-Aloul et al, 2004) lead to a high level of anxiety among the CF physicians who were keen to treat them as effectively as possible. Limited understanding of their phenotypic diversity and complex susceptibility patterns *at that time* resulted in a unit policy that subjected our patients to repeated courses of aminoglycosides and / or polymyxins due to the antibiograms often, but not always, indicating resistance of LES to other antipseudomonal antibiotics. However, the observation of several cases of acute renal failure related to this antibiotic combination between 2000 and 2004 challenged this dogma (chapter 5). Subsequently, evidence emerged indiacting that CF patients improve on antibiotic combinations even when their *P. aeruginosa* isolates appear resistant *in vitro* to one or all antibiotics in that combination (Smith et al, 2003; Aaron et al, 2005). The validity of conventional *in vitro* susceptibility testing itself is questioned in the context of CF: multiple identical looking colonies of *P. aeruginosa* isolated from the same sputum sample can give different antibiograms. Routine susceptibility test results are not reproducible and underestimate resistance (Foweraker et al, 2005). Synergy testing may help circumvent some of these challenges but cost, restricted availability, delayed results (often not available until the patient has been discharged from hospital) have all limited its usefulness. Furthermore, *in vitro* antibiotic synergy results on isolates from the same sputum sample can vary depending on the methodology used. A recent report described poor correlation between the results of the different methods of synergy testing (checkerboard, time-kill curve and multiple combination bactericidal testing [MCBT]), and none of these methods was capable of predicting the clinical response to treatment in all patients studied (Foweraker et al, 2009). Lack of correlation between *in vitro* susceptibility results and clinical and microbiological outcomes lead to the CF unit antibiotic policy becoming less rigid: whilst sensitivity patterns on the antibiogram are still considered, the choice of

antibiotic combination is tempered against the individual patient's previous experience with success or failure of certain therapy, their background of antibiotic allergy or intolerance as well as their personal preference (expressed by some patients more forcefully than others!).

Also of note is the dissociation between acute tubular injury as witnessed by enzymuria and proteinuria and the improvement in measured CCl after a standard 14 day IV antibiotic therapy of an acute pulmonary exacerbation (chapters 7, 8 & 9). This highlights the complex interaction between circulatory, oncotic and local paracrine forces at the glomerular and tubular levels. GFR and tubular absorptive/concentrating capacity could be measuring two different, but closely interdependent aspects of renal function, relative injury to which, in the face of different nephrotoxins, is also unclear. CF patients at high risk of cumulative drug injury should be carefully monitored for tubular function as well as glomerular performance.

10.5 Recommendations

Based on the literature review and original work contained in this thesis, I would recommend that CF health care providers adopt the following reno-protective strategies:

- Alternate antibiotic classes integrating aminoglycoside holidays
- If aminoglycosides have to be used:
    - Consider once vs multiple daily dosing regimens
    - Choose less toxic aminoglycosides, if possible
    - Apply regular drug level monitoring
    - Employ vigilance with concurrent nephrotoxic agents
    - Pay attention to hydration

- Consider targeted drug delivery with nebulised aminoglycosides to treat acute exacerbations
- Consider adding fosfomycin to aminoglycoside therapy, to help protect tubular cell lysosomal membrane integrity

10.6 Suggestions for future research

10.6.1 Possible different approaches to the present study

If I had a chance to start all over again, things I would do differently:

- In the absence of a more robust and validated tool, I would endeavour to apply radioactive agent clearance, as the gold standard reference for assessing prevalence of renal dysfunction and monitoring long term changes with therapy. This could be justified in stable outpatients to gauge their GFR more accurately and remove the confounders surrounding timed urine collections. It is less applicable however in the acute setting when patients are admitted unwell and at times toxic with an exacerbation. It would not have been possible or ethical to justify its use in the prospective studies relating to drug treatment of acute exacerbations (chapters 8 & 9). Instead, urine markers were more readily measured in this situation as they did not add much to the burden of care the patients endured.

- I would choose a different marker of tubular proteinuria: there are now commercial assays for measuring urine concentrations of several low molecular weight serum proteins which leak in tubular disease because of incomplete tubular reabsorption. Retinol binding protein and glutathione transferases, for example, are easier to assay than β2M as their structure is more stable and independent of urine pH (Trof et al, 2006; Bagshaw et al, 2007; Coscia et al, 2008). In fact some commercial laboratories now supply

retinol binding globulin for research purposes, extracted from human urine from patients with chronic renal tubular proteinuria.

- Albumin/creatinine ratio instead of measurement of 24-hour proteinuria? Very little is published on the measurement of proteinuria in CF urine. Drawing any conclusion is difficult due to the inconsistent reporting, lack of clarity regarding laboratory techniques and what exactly was being measured. Thresholds of normality depend on whether one is measuring urine microalbumin, albumin, protein, albumin/creatinine ratio or protein/creatinine ratio. Most hospital biochemistry laboratories now offer, as routine service, measurement of albumin/creatinine ratio (ACR). For adults without diabetes, significant proteinuria is an ACR of 30 mg/mmol or more. In diabetics, microalbuminuria is measured and the normal value is <2.5 mg/mmol in males and <3.5 mg/mmol in females (Hofmann and Guder, 1989). Microalbumin may still be significant in other patient groups. There is clearly a continuum between microalbuminuria and albuminuria/proteinuria.

10.6.2 Validation of new assays of renal function?

10.6.2.1 Cystatin C

Cystatin C, a cysteine protease inhibitor, is a non-glycosylated low molecular weight protein produced by all nucleated cells. Cystatin C is freely filtered by the glomerulus before being metabolized by the proximal tubular epithelial cells; that is, once cystatin C is filtered it does not re-enter the circulation in its original form. It is theoretically an ideal endogenous marker for estimating GFR as serum cystatin C is mainly determined by glomerular filtration and is not affected by muscle mass, sex, or age (Mussap and Plebani, 2004).

As a simple blood test, measurement of serum cystatin C would offer significant advantages over standard cumbersome and/or invasive methods for GFR measurement if it correlated well. Serum concentrations of cystatin C are inversely related to its clearance, so generally the reciprocal value of the serum cystatin C sample is used as the comparator. Many, but not all, studies have shown that serum cystatin C correlates more closely with the reference method used including ^{51}Cr-EDTA, ^{125}I-labeled iothalamate clearance, iohexol, inulin clearance, and ^{99}mTc- DTPA than SCr (Dharnidharka et al, 2002). Although cystatin C is a promising candidate as an indirect marker for GFR not all studies have shown that it is superior to using calculated CCl. In a pooled analysis by Stevens *et al* (Stevens et al, 2008) they concluded that serum cystatin C levels were nearly as accurate as SCr levels that had been adjusted for age, sex, and race. A literature review (Zahran et al, 2007) identified 43 studies that compared the performance of cystatin C and SCr for estimating GFR. Fourteen of these studies were in patients who had received a kidney transplant and 70% of the studies favoured cystatin C over SCr. The other 29 studies were in patients with identified kidney disease: none showed superiority of SCr over serum cystatin C and, 60% of them favoured serum cystatin C. A further 11 studies were identified that directly compared SCr-based equations with cystatin-C-based equations: cystatin C performed better. This review showed that cystatin C was not uniformly better in all patient groups but it may be a preferred option in certain patient populations. In a study not included in the above review, Perkins *et al* (Perkins et al, 2005) monitored renal function in patients with type 2 diabetes over 4 years. Cystatin C was compared with iothalamate clearance. Perkins *et al* concluded that cystatin C could accurately detect trends in renal function over time in this patient group. This study may be relevant as many adult CF patients also have diabetes. Halacova *et al* (Halacova et al, 2008) used cystatin C to assess GFR in patients with CF who were treated with IV amikacin. Cystatin C appeared to be a better marker of GFR than either using SCr or CCl (calculated using a pharmacokinetic computer programme, MW/PHARM version 3.3.0). Recently, the potential role of cystatin C has been specifically explored in patients with CF by Beringer *et al* (Beringer et al, 2009). Nineteen patients with CF had their renal

function measured using iothalamate clearance as the gold standard. Simultaneously, blood samples were taken to measure creatinine and cystatin C levels. Cystatin C was not only more precise at estimating renal function but also had greater sensitivity and specificity when compared to two creatinine-based equations (CGF and aMDRD). They concluded that there might be a role for cystatin C in measuring renal function in patients with CF.

The advantage of using cystatin C over conventional equations is that no other patient data are required.

10.6.2.2 Tobramcyin clearance

It has been hypothesized that tobramycin clearance itself could be used as a predictor of renal function in patients with CF. Smith *et al* (Smith et al, 2006) reported the role of tobramycin clearance as an indirect measure of GFR in nine children with CF. Unfortunately, they found that there was little correlation between GFR measured using an isotopic technique and the values for tobramycin clearance determined using the ALADDIN method for therapeutic drug monitoring. Similarly, Levy et al (Levy et al, 1984) found no correlation between measured creatinine clearance and tobramycin renal clearance. This could be due to a variety of factors including the small numbers of patients in these studies and the fact that approximately 20% of tobramycin is reabsorbed after filtration thus possibly explaining the observation that the predicted values of tobramycin clearance were lower than the measured values of GFR (Matthews et al, 2004). In contrast, tobramycin clearance was found to correlate well with measured creatinine clearance in 18 adult patients with CF (Touw et al, 1994). The evidence is therefore conflicting over the actual relationship of tobramycin clearance to GFR. Many centres now use computerized programmes for therapeutic drug monitoring of tobramycin and therefore would have access to tobramycin clearance values for each admission and the capacity to monitor changes in those values. As this information is readily available, it would seem to be an area that could be

investigated further to determine the true value of tobramycin clearance as an indirect measure of GFR.

10.6.3 Interaction between CFRD and renal function

I did not find a significant interaction between CFRD and antibiotic-related kidney toxicity in the regressional analysis of the cross sectional data presented in chapter 4. However, In the 36 patients with CFRD included in the study by Etherington *et al* (Etherington et al, 2007), baseline urinary NAG levels were elevated at the start of IV tobramycin treatment in 27 patients and remained so at their routine follow-up clinic appointment. This suggests that patients with CFRD may already have renal impairment. Mechanisms of this are currently unknown and deserve further attention. The improved survival of CF patients means they live longer with CFRD, increasing the risk of developing microangiopathic complications akin to those seen in the non CF diabetics (Sullivan and Denning, 1989; Westall et al, 2004). In the absence of histological confirmation, microalbuminuria is a sensitive marker of early diabetic nephropathy. In a case control study, a higher prevalence of microalbuminuria in the CFRD group compared with non-CF type 1 insulin-dependent diabetics was described, even after correcting for blood pressure and level of glycaemic control (van den Berg et al, 2008) but interestingly Etherington *et al* found a normal albumin/creatinine ratio in those with and without CFRD (Etherington et al, 2007). This may reflect the influence of other, as yet undetermined, CF-related factors on renal function which require further study.

10.6.4 Adequately powered multicentre studies

Collaborations are essential to retest the concepts presented in this thesis with sufficient power. As mentioned in chapter 8, 80 to 130 patients are required in a parallel group study to show equivalence between two antibiotic treatment strategies with 80% power, using FEV1 % predicted as the primary end point and urinary NAG as the secondary. As experience is shared and data accumulates, it is

conceivable that sufficient data will be available to define reference ranges for the CF population with respect to GFR, tubular enzymes and cystatin C. It may even be possible to draft a CF-specific equation for GFR estimation replacing generic ones such as CGF. This would allow for a non invasive tool to help deliver individualised patient-tailored therapy.

References

Aaron SD, Ferris W, Ramotar K, Vandemheen K, Chan F, Saginur R. Single and combination antibiotic susceptibilities of planktonic, adherent, and biofilm-grown *Pseudomonas aeruginosa* isolates cultured from sputa of adults with cystic fibrosis. *J Clin Microbiol* 2002; **40**: 4172-4179.

Aaron SD et al. Combination antibiotic susceptibility testing to treat exacerbations of cystic fibrosis associated with multiresistant bacteria: a randomised, double-blind, controlled clinical trial. *Lancet* 2005; **366**: 463-471.

Adeboyeku DU, Burgess JC, Hodson ME. Antibiotic sensitivity and clinical outcome of acute respiratory exacerbations in adult CF patients colonised with *P. aeruginosa. Paediatr Pulmonol* 1999; **suppl 19**: 264.

Agarwal R, Nicar M. Comparative analysis of formulas used to predict creatinine clearance. *J Am Soc Nephrol* 1994; **5**: 386 (Abstract).

Akbar A, Rees JH, Nyamugunduru G, English MW, Spencer DA, Weller PH. Aminoglycoside-associated hypomagnesaemia in children with cystic fibrosis. *Acta Paediatr* 1999; **88**: 783-785.

Alcanatra P, Goncalves F, Moreira C, Gonzalez M. Assessment of glomerular filtration rate in a hospital population: Comparison of two methods. *Acta Med Port* 1998; **11**: 767-772.

Alexopoulos E. Drug-induced acute interstitial nephritis. *Ren Fail* 1998; **20**: 809-819.

Alothman GA, Alsaadi MM, Ho BL, Ho SL, Dupuis A, Corey M, Coates AL. Evaluation of bronchial constriction in children with cystic fibrosis after inhaling two different preparations of tobramycin. *Chest* 2002; **122**: 930-934.

Andersen HU, Lanng S, Pressler T, Laugesen CS, Mathiesen ER. Cystic fibrosis-related diabetes: the presence of microvascular diabetes complications. *Diabetes Care* 2006; **29**: 2660-2663.

Anderson MP, Welsh MJ. Regulation by ATP and ADP of CFTR chloride channels that contain mutant nucleotide-binding domains. *Science* 1992; **257**: 1701-1704.

Anwar H, Strap JL, Costerton JW. Establishment of aging biofilms: possible mechanism of bacterial resistance to antimicrobial therapy. *Antimicrob Agents Chemother* 1992; **36**: 1347-1351.

Armstrong DS, Grimwood K, Carzino R, Carlin JB, Olinsky A, Phelan PD. Lower respiratory infection and inflammation in infants with newly diagnosed cystic fibrosis. *BMJ* 1995; **310**: 1571-1572.

Armstrong DS, Grimwood K, Carlin JB, Carzino R, Olinsky A, Phelan PD. Bronchoalveolar lavage or oropharyngeal cultures to identify lower respiratory pathogens in infants with cystic fibrosis. *Pediatr Pulmonol* 1996; **21**: 267-275.

Armstrong DS, Nixon GM, Carzino R, Bigham A, Carlin JB, Robins-Browne RM, Grimwood K. Detection of a widespread clone of *Pseudomonas aeruginosa* in a pediatric cystic fibrosis clinic. *Am J Respir Crit Care Med* 2002; **166**: 983-987.

Assael BM, Marra G, Tirelli AS, Cavanna G, Claris Appiani A, Giunta A, Amoretti M, Milani S. Renal function in cystic fibrosis. *Int J Pediatr Nephrol* 1986; **7**: 213-216.

Bagshaw SM, Langenberg C, Haase M, Wan L, May CN, Bellomo R. Urinary biomarkers in septic acute kidney injury. *Intensive Care Med* 2007; **33**: 1285-1296.

Baker R. Renal disease. In: Clinical Medicine, Fifth Edition. Kumar P, Clark M, eds. New York: W.B. Saunders, 2002: 587-666.

Balla AA, Salah AM, Abdalmotaal E, Hoppe B, Bongartz D, Kessler T, Hesse A. N-acetyl-beta-D-glucosaminidase excretion in healthy children and in pediatric patients with urolithiasis. *World J Urol* 1998; **16**: 413-416.

Bannister K, Field M. Clinical physiology of the kidney. In: Oxford Textbook of Medicine, Third Edition. Weatherall D, Ledingham J, Warrell D, eds. New York: Oxford University Press, 1996: 3103-3115.

Barasch J, Kiss B, Prince A, Saiman L, Gruenert D, al-Awqati Q. Defective acidification of intracellular organelles in cystic fibrosis. *Nature* 1991; **352**: 70-73.

Bargon J, Dauletbaev N, Kohler B, Wolf M, Posselt HG, Wagner TO. Prophylactic antibiotic therapy is associated with an increased prevalence of Aspergillus colonization in adult cystic fibrosis patients. *Respir Med* 1999; **93**: 835-838.

Barilla DE, Tolentino R, Kaplan RA, Pak CY. Selective effects of thiazide on intestinal absorption of calcium and adsorptive and renal hypercalciurias. *Metabolism* 1978; **27**: 125-131.

Barriere H, Belfodil R, Rubera I, Tauc M, Poujeol C, Bidet M, Poujeol P. CFTR null mutation altered cAMP-sensitive and swelling-activated Cl^- currents in primary cultures of mouse nephron. *Am J Physiol Renal Physiol* 2003; **284**: F796-811.

Bates CM, Baum M, Quigley R. Cystic fibrosis presenting with hypokalemia and metabolic alkalosis in a previously healthy adolescent. *J Am Soc Nephrol* 1997; **8**: 352-355.

Bauer JH, Brooks CS, Burch RN. Renal function studies in man with advanced renal insufficiency. *Am J Kidney Dis* 1982a; **2**: 30-35.

Bauer JH, Brooks CS, Burch RN. Clinical appraisal of creatinine clearance as a measurement of glomerular filtration rate. *Am J Kidney Dis* 1982b; **2**: 337-346.

Bennett WM. Aminoglycoside nephrotoxicity. *Nephron* 1983; **35**: 73-77.

Bennett WM. Drug interactions and consequences of sodium restriction. *Am J Clin Nutr* 1997; **65**: 678S-681S.

Bens M, Van Huyen JP, Cluzeaud F, Teulon J, Vandewalle A. CFTR disruption impairs cAMP-dependent Cl(-) secretion in primary cultures of mouse cortical collecting ducts. *Am J Physiol Renal Physiol* 2001; **281**: F434-442.

Berger HA, Anderson MP, Gregory RJ, Thompson S, Howard PW, Maurer RA, Mulligan R, Smith AE, Welsh MJ. Identification and regulation of the cystic fibrosis transmembrane conductance regulator-generated chloride channel. *J Clin Invest* 1991; **88**: 1422-1431.

Beringer PM, Hidayat L, Heed A, Zheng L, Owens H, Benitez D, Rao AP. GFR estimates using cystatin C are superior to serum creatinine in adult patients with cystic fibrosis. *J Cyst Fibros* 2009; **8**: 19-25.

Bernard AM, Moreau D, Lauwerys R. Comparison of retinol-binding protein and beta 2-microglobulin determination in urine for the early detection of tubular proteinuria. *Clin Chim Acta* 1982; **126**: 1-7.

Berry CA, Rector FCJ. Renal Transport of glucose, amino acids, sodium, chloride and water. In: The Kidney, fourth Edition. Brenner BM, Rector FCJ, eds. Philadelphia: Saunders, 1991: 245-282

Bertenshaw C, Watson AR, Lewis S, Smyth A. Survey of acute renal failure in patients with cystic fibrosis in the UK. *Thorax* 2007; **62**: 541-545.

Bilton D, Henig N, Morrissey B, Gotfried M. Addition of inhaled tobramycin to ciprofloxacin for acute exacerbations of *Pseudomonas aeruginosa* infection in adult bronchiectasis. *Chest* 2006; **130**: 1503-1510.

Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986; **1**: 307-310.

Blumsohn A, Morris BW, Griffiths H, Ramsey CF. Stability of beta 2-microglobulin and retinol binding protein at different values of pH and temperature in normal and pathological urine. *Clin Chim Acta* 1991; **195**: 133-137.

Bohles H, Michalk D. Is there a risk for kidney stone formation in cystic fibrosis? *Helv Paediatr Acta* 1982; **37**: 267-272.

Bosomworth MP, Aparicio SR, Hay AW. Urine N-acetyl-beta-D-glucosaminidase--a marker of tubular damage? *Nephrol Dial Transplant* 1999; **14**: 620-626.

Bosso JA, Liptak CA, Seilheimer DK, Harrison GM. Toxicity of colistin in cystic fibrosis patients. *DICP* 1991; **25**: 1168-1170.

Boyle MP. Adult cystic fibrosis. *JAMA* 2007; **298**: 1787-1793.

Branten AJ, Wetzels JF. Influence of albumin infusion on the urinary excretion of beta2-microglobulin in patients with proteinuria. *Nephron* 1999; **81**: 329-333.

Breen L, Aswani N. Elective versus symptomatic intravenous antibiotic therapy for cystic fibrosis. *Cochrane Database Syst Rev* 2001: CD002767.

Brennan AL, Geddes DM, Gyi KM, Baker EH. Clinical importance of cystic fibrosis-related diabetes. *J Cyst Fibros* 2004; **3**: 209-222.

Broekroelofs J, Navis GJ, Stegeman CA, van der Bij W, de Boer WJ, de Zeeuw D, de Jong PE. Long-term renal outcome after lung transplantation is predicted by the 1-month postoperative renal function loss. *Transplantation* 2000; **69**: 1624-1628.

Burden R, Tomson C. Identification, management and referral of adults with chronic kidney disease: concise guidelines. *Clin Med* 2005; **5**: 635-642.

Burkle WS. Is tobramycin less nephrotoxic than gentamicin? *Clin Pharm* 1986; **5**: 514-516.

Burns JL, Emerson J, Stapp JR, Yim DL, Krzewinski J, Louden L, Ramsey BW, Clausen CR. Microbiology of sputum from patients at cystic fibrosis centers in the United States. *Clin Infect Dis* 1998; **27**: 158-163.

Burns JL, Van Dalfsen JM, Shawar RM, Otto KL, Garber RL, Quan JM, Montgomery AB, Albers GM, Ramsey BW, Smith AL. Effect of chronic intermittent administration of inhaled tobramycin on respiratory microbial flora in patients with cystic fibrosis. *J Infect Dis* 1999; **179**: 1190-1196.

Burrows JA, Nissen LM, Kirkpatrick CM, Bell SC. Beta-lactam allergy in adults with cystic fibrosis. *J Cyst Fibros* 2007; **6**: 297-303.

Bye MR, Ewig JM, Quittell LM. Cystic fibrosis. *Lung* 1994; **172**: 251-270.

Cameron J, Greger R. Renal function and testing function. In: Oxford Textbook of Clinical Nephrology, second Edition. Davidson A, Cameron J, Grunfeld J-P, Kerr D, Ritz E, Winearls C, eds. New York: Oxford University Press, 1997: 39-69

Canales M, Youssef P, Spong R, Ishani A, Savik K, Hertz M, Ibrahim HN. Predictors of chronic kidney disease in long-term survivors of lung and heart-lung transplantation. *Am J Transplant* 2006; **6**: 2157-2163.

Cheng K, Smyth RL, Govan JR, Doherty C, Winstanley C, Denning N, Heaf DP, van Saene H, Hart CA. Spread of beta-lactam-resistant *Pseudomonas aeruginosa* in a cystic fibrosis clinic. *Lancet* 1996; **348**: 639-642.

Chidekel AS, Dolan TF, Jr. Cystic fibrosis and calcium oxalate nephrolithiasis. *Yale J Biol Med* 1996; **69**: 317-321.

Ciofu O, Giwercman B, Pedersen SS, Hoiby N. Development of antibiotic resistance in *Pseudomonas aeruginosa* during two decades of antipseudomonal treatment at the Danish CF Center. *APMIS* 1994; **102**: 674-680.

Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Fifteenth Informational Supplement M100-S15. CLSI 2005. Wayne PA, USA.

Cochran M, St John A. A comparison between estimates of GFR using [99mTc]DTPA clearance and the approximation of Cockcroft and Gault. *Aust N Z J Med* 1993; **23**: 494-497.

Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron* 1976; **16**: 31-41.

Conway SP. Evidence for using nebulised antibiotics in cystic fibrosis. *Arch Dis Child* 1999; **80**: 307-309.

Conway SP, Brownlee KG, Denton M, Peckham DG. Antibiotic treatment of multidrug-resistant organisms in cystic fibrosis. *Am J Respir Med* 2003; **2**: 321-332.

Conway SP, Pond MN, Watson A, Etherington C, Robey HL, Goldman MH. Intravenous colistin sulphomethate in acute respiratory exacerbations in adult patients with cystic fibrosis. *Thorax* 1997; **52**: 987-993.

Conway SP, Etherington C, Munday J, Goldman MH, Strong JJ, Wootton M. Safety and tolerability of bolus intravenous colistin in acute respiratory exacerbations in adults with cystic fibrosis. *Ann Pharmacother* 2000; **34**: 1238-1242.

Coscia A, Maiorca D, Martano C, Rossi C, Appino I, Cirina P, Alessi D, Fabris C. Use of netilmicin once or twice daily in preterm newborns: evaluation of nephrotoxicity by urinary alpha1-microglobulin and retinol binding protein. *J Chemother* 2008; **20**: 324-326.

Coulthard KP, Peckham DG, Conway SP, Smith CA, Bell J, Turnidge J. Therapeutic drug monitoring of once daily tobramycin in cystic fibrosis--caution with trough concentrations. *J Cyst Fibros* 2007; **6**: 125-130.

Courcol RJ, Martin GR. In-vitro activity of the combination of ceftriaxone and fosfomycin against staphylococci. *J Antimicrob Chemother* 1987; **19**: 276-278.

Crawford I, Maloney PC, Zeitlin PL, Guggino WB, Hyde SC, Turley H, Gatter KC, Harris A, Higgins CF. Immunocytochemical localization of the cystic fibrosis gene product CFTR. *Proc Natl Acad Sci U S A* 1991; **88**: 9262-9266.

Cronberg S, Nordstrom L, Ringberg H. Prediction of creatinine clearance by several methods in patients with severe infections. *Eur J Clin Pharmacol* 1992; **42**: 193-195.

Cullen AR, Cannon CL, Mark EJ, Colin AA. *Mycobacterium abscessus* infection in cystic fibrosis. Colonization or infection? *Am J Respir Crit Care Med* 2000; **161**: 641-645.

Cystic Fibrosis Trust. *Pseudomonas aeruginosa* infection in people with cystic fibrosis: suggestions for prevention and infection control. Report of the Cystic Fibrosis Trust Control of Infection Group 2001 (First Edition), Kent, UK.

Cystic Fibrosis Trust. Antibiotic treatment for cystic fibrosis. Cystic Fibrosis Trust Antibiotic Group 2002 (Second Edition), London, UK.

Dakin C, Henry RL, Field P, Morton J. Defining an exacerbation of pulmonary disease in cystic fibrosis. *Pediatr Pulmonol* 2001; **31**: 436-442.

Davey PG, Geddes AM, Cowley DM. Study of alanine aminopeptidase excretion as a test of gentamicin nephrotoxicity. *J Antimicrob Chemother* 1983; **11**: 455-465.

Davey RJ, Peckham D, Etherington C, Denton M, Conway S. Antibiotic resistance and clinical outcome following intravenous antibiotic therapy for respiratory exacerbations in cystic fibrosis. In: Abstract book, XIIIth International CF Congress, Stockholm, 2000: A409.

Davies JC. *Pseudomonas aeruginosa* in cystic fibrosis: pathogenesis and persistence. *Paediatr Respir Rev* 2002; **3**: 128-134.

Davila E, Gardner LB. Clinical value of the creatinine clearance before the administration of chemotherapy with cisplatin. *Cancer* 1987; **60**: 161-164.

Davis PB, Drumm M, Konstan MW. Cystic fibrosis. *Am J Respir Crit Care Med* 1996; **154**: 1229-1256.

Daza R, Moreno-Lopez M, Damaso D. Interactions of fosfomycin with other antibiotics. *Chemotherapy* 1977; **23 Suppl 1**: 86-92.

De Broe ME, Giuliano RA, Verpooten GA. Aminoglycoside nephrotoxicity: mechanism and prevention. *Adv Exp Med Biol* 1989; **252**: 233-245.

de Cueto M, Lopez L, Hernandez JR, Morillo C, Pascual A. In vitro activity of fosfomycin against extended-spectrum-beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*: comparison of susceptibility testing procedures. *Antimicrob Agents Chemother* 2006; **50**: 368-370.

Denton M, Todd NJ, Littlewood JM. Role of anti-pseudomonal antibiotics in the emergence of *Stenotrophomonas maltophilia* in cystic fibrosis patients. *Eur J Clin Microbiol Infect Dis* 1996a; **15**: 402-405.

Denton M, Littlewood JM, Brownlee KG, Conway SP, Todd NJ. Spread of beta-lactam-resistant *Pseudomonas aeruginosa* in a cystic fibrosis unit. *Lancet* 1996b; **348**: 1596-1597.

Denton M, Kerr K, Mooney L, Keer V, Rajgopal A, Brownlee K, Arundel P, Conway S. Transmission of colistin-resistant *Pseudomonas aeruginosa* between patients attending a pediatric cystic fibrosis center. *Pediatr Pulmonol* 2002; **34**: 257-261.

Devuyst O, Burrow CR, Schwiebert EM, Guggino WB, Wilson PD. Developmental regulation of CFTR expression during human nephrogenesis. *Am J Physiol* 1996; **271**: F723-735.

Dharnidharka VR, Kwon C, Stevens G. Serum cystatin C is superior to serum creatinine as a marker of kidney function: a meta-analysis. *Am J Kidney Dis* 2002; **40**: 221-226.

Diadyk AI, Siniachenko OV, Shtoda LA, Siniachenko T, Khomenko MV, Iarovaia NF, Zviagina TV. The beta 2-microglobulin content of the blood serum and urine in patients with primary chronic and lupus glomerulonephritis. *Vrach Delo* 1991: 61-64.

Diener U, Knoll E, Langer B, Rautenstrauch H, Ratge D, Wisser H. Urinary excretion of N-acetyl-beta-D-glucosaminidase and alanine aminopeptidase in patients receiving amikacin or cis-platinum. *Clin Chim Acta* 1981; **112**: 149-157.

Djelidi S, Fay M, Cluzeaud F, Thomas-Soumarmon A, Bonvalet JP, Farman N, Blot-Chabaud M. Vasopressin stimulates long-term net chloride secretion in cortical collecting duct cells. *FEBS Lett* 1999; **460**: 533-538.

Dodge JA, Lewis PA, Stanton M, Wilsher J. Cystic fibrosis mortality and survival in the UK: 1947-2003. *Eur Respir J* 2007; **29**: 522-526.

Donaldson MD, Chambers RE, Woolridge MW, Whicher JT. Stability of alpha 1-microglobulin, beta 2-microglobulin and retinol binding protein in urine. *Clin Chim Acta* 1989; **179**: 73-77.

Donckerwolcke RA, van Diemen-Steenvoorde R, van der Laag J, Koomans HA, Boer WH. Impaired diluting segment chloride reabsorption in patients with cystic fibrosis. *Child Nephrol Urol* 1992; **12**: 186-191.

dos Santos AC, Colacciopo S, Dal Bo CM, dos Santos NA. Occupational exposure to lead, kidney function tests, and blood pressure. *Am J Ind Med* 1994; **26**: 635-643.

Drew J, Watson AR, Smyth A. Acute renal failure and cystic fibrosis. *Arch Dis Child* 2003; **88**: 646.

Drew J, Watson A, Evans J, Smyth A. Antibiotics and acute renal failure in children with cystic fibrosis. . *Paediatr Perinat Drug Ther* 2002; **5**: 65–67.

Duong Van Huyen J, Bens M, Vandewalle A. Differential effects of aldosterone and vasopressin on chloride fluxes in transimmortalized mouse cortical collecting duct cells. *J Membr Biol* 1998; **164**: 79-90.

Edson RS, Brey RH, McDonald TJ, Terrell CL, McCarthy JT, Thibert JM. Vestibular toxicity due to inhaled tobramycin in a patient with renal insufficiency. *Mayo Clin Proc* 2004; **79**: 1185-1191.

Eisenberg J, Pepe M, Williams-Warren J, Vasiliev M, Montgomery AB, Smith AL, Ramsey BW. A comparison of peak sputum tobramycin concentration in patients with cystic fibrosis using jet and ultrasonic nebulizer systems. Aerosolized Tobramycin Study Group. *Chest* 1997; **111**: 955-962.

Elborn JS, Prescott RJ, Stack BH, Goodchild MC, Bates J, Pantin C, Ali N, Shale DJ, Crane M. Elective versus symptomatic antibiotic treatment in cystic fibrosis patients with chronic Pseudomonas infection of the lungs. *Thorax* 2000; **55**: 355-358.

Emerson J, Rosenfeld M, McNamara S, Ramsey B, Gibson RL. *Pseudomonas aeruginosa* and other predictors of mortality and morbidity in young children with cystic fibrosis. *Pediatr Pulmonol* 2002; **34**: 91-100.

Etherington C, Bosomworth M, Clifton I, Peckham DG, Conway SP. Measurement of urinary N-acetyl-b-D-glucosaminidase in adult patients with cystic fibrosis: before, during and after treatment with intravenous antibiotics. *J Cyst Fibros* 2007; **6**: 67-73.

Etherington C, Hall M, Conway S, Peckham D, Denton M. Clinical impact of reducing routine susceptibility testing in chronic Pseudomonas aeruginosa infections in cystic fibrosis. *J Antimicrob Chemother* 2008; **61**: 425-427.

Falagas ME, Kasiakou SK. Toxicity of polymyxins: a systematic review of the evidence from old and recent studies. *Crit Care* 2006; **10**: R27.

Falagas ME, Fragoulis KN, Kasiakou SK, Sermaidis GJ, Michalopoulos A. Nephrotoxicity of intravenous colistin: a prospective evaluation. *Int J Antimicrob Agents* 2005; **26**: 504-507.

Falagas ME, Sideri G, Vouloumanou EK, Papadatos JH, Kafetzis DA. Intravenous colistimethate (colistin) use in critically ill children without cystic fibrosis. *Pediatr Infect Dis J* 2009; **28**: 123-127.

Farago E, Kiss IJ, Nabradi Z. Serum and lung tissue levels of fosfomycin in humans. *Int J Clin Pharmacol Ther Toxicol* 1980; **18**: 554-558.

Figueredo VM, Neu HC. Synergy of ciprofloxacin with fosfomycin in vitro against Pseudomonas isolates from patients with cystic fibrosis. *J Antimicrob Chemother* 1988; **22**: 41-50.

Finnegan MJ, Hinchcliffe J, Russell-Jones D, Neill S, Sheffield E, Jayne D, Wise A, Hodson ME. Vasculitis complicating cystic fibrosis. *Q J Med* 1989; **72**: 609-621.

Forsgren A, Walder M. Antimicrobial activity of fosfomycin in vitro. *J Antimicrob Chemother* 1983; **11**: 467-471.

Foskett JK. ClC and CFTR chloride channel gating. *Annu Rev Physiol* 1998; **60**: 689-717.

Foweraker JE, Laughton CR, Brown DF, Bilton D. Phenotypic variability of *Pseudomonas aeruginosa* in sputa from patients with acute infective exacerbation of cystic fibrosis and its impact on the validity of antimicrobial susceptibility testing. *J Antimicrob Chemother* 2005; **55**: 921-927.

Foweraker JE, Laughton CR, Brown DF, Bilton D. Comparison of methods to test antibiotic combinations against heterogeneous populations of multiresistant *Pseudomonas aeruginosa* from patients with acute infective exacerbations in cystic fibrosis. *Antimicrob Agents Chemother* 2009; **53**: 4809-4815.

Frederiksen B, Koch C, Hoiby N. Antibiotic treatment of initial colonization with *Pseudomonas aeruginosa* postpones chronic infection and prevents deterioration of pulmonary function in cystic fibrosis. *Pediatr Pulmonol* 1997; **23**: 330-335.

Frederiksen B, Lanng S, Koch C, Hoiby N. Improved survival in the Danish center-treated cystic fibrosis patients: results of aggressive treatment. *Pediatr Pulmonol* 1996; **21**: 153-158.

Fujita K. An effect of fosfomycin on the pharmacokinetics of amikacin. *J Antibiot (Tokyo)* 1984; **37**: 408-412.

Fujita K, Fujita HM, Aso Y. Protective effect of fosfomycin against renal accumulation of aminoglycoside antibiotics. *Jpn J Antibiot* 1983; **36**: 3392-3394.

Gaffney K, Gibbons D, Keogh B, FitzGerald MX. Amyloidosis complicating cystic fibrosis. *Thorax* 1993; **48**: 949-950.

Gan KH, Veeze HJ, van den Ouweland AM, Halley DJ, Scheffer H, van der Hout A, Overbeek SE, de Jongste JC, Bakker W, Heijerman HG. A cystic fibrosis mutation associated with mild lung disease. *N Engl J Med* 1995; **333**: 95-99.

Gatta A, Bazzerla G, Amodio P, Menon F, Angeli P, Schiaffino E, Schmid C. Detection of the early steps of cadmium nephropathy--comparison of light- and electron-microscopical patterns with the urinary enzymes excretion. An experimental study. *Nephron* 1989; **51**: 20-24.

Geller DE, Pitlick WH, Nardella PA, Tracewell WG, Ramsey BW. Pharmacokinetics and bioavailability of aerosolized tobramycin in cystic fibrosis. *Chest* 2002; **122**: 219-226.

Gibey R, Dupond JL, Alber D, Leconte des Floris R, Henry JC. Predictive value of urinary N-acetyl-beta-D-glucosaminidase (NAG), alanine-aminopeptidase (AAP) and beta-2-microglobulin (beta 2M) in evaluating nephrotoxicity of gentamicin. *Clin Chim Acta* 1981; **116**: 25-34.

Gibney EM, Goldfarb DS. The association of nephrolithiasis with cystic fibrosis. *Am J Kidney Dis* 2003; **42**: 1-11.

Gibson RL, Burns JL, Ramsey BW. Pathophysiology and management of pulmonary infections in cystic fibrosis. *Am J Respir Crit Care Med* 2003a; **168**: 918-951.

Gibson RL, Emerson J, McNamara S, Burns JL, Rosenfeld M, Yunker A, Hamblett N, Accurso F, Dovey M, Hiatt P, Konstan MW, Moss R, Retsch-Bogart G, Wagener J, Waltz D, Wilmott R, Zeitlin PL, Ramsey B. Significant microbiological effect of inhaled tobramycin in young children with cystic fibrosis. *Am J Respir Crit Care Med* 2003b; **167**: 841-849.

Giovannetti S, Barsotti G. In defense of creatinine clearance. *Nephron* 1991; **59**: 11-14.

Glass S, Plant ND, Spencer DA. The effects of intravenous tobramycin on renal tubular function in children with cystic fibrosis. *J Cyst Fibros* 2005; **4**: 221-225.

Godson C, Ryan MP, O'Halloran D, Bourke S, Brady HR, FitzGerald MX. Investigation of aminoglycoside nephrotoxicity in cystic fibrosis patients. *Scand J Gastroenterol Suppl* 1988; **143**: 70-73.

Govan JR. Insights into cystic fibrosis microbiology from the European tobramycin trial in cystic fibrosis. *J Cyst Fibros* 2002; **1**: 203-208.

Groeneweg M, Tan S, Boot AM, de Jongste JC, Bouquet J, Sinaasappel M. Assessment of nutritional status in children with cystic fibrosis: conventional anthropometry and bioelectrical impedance analysis. A cross-sectional study in Dutch patients. *J Cyst Fibros* 2002; **1**: 276-280.

Grunden JW, Fisher KA. Lovastatin-induced rhabdomyolysis possibly associated with clarithromycin and azithromycin. *Ann Pharmacother* 1997; **31**: 859-863.

Guasch A, Cua M, Mitch WE. Early detection and the course of glomerular injury in patients with sickle cell anemia. *Kidney Int* 1996; **49**: 786-791.

Gutknecht DR. Kidney stones and cystic fibrosis. *Am J Med* 2001; **111**: 83.

Halacova M, Kotaska K, Kukacka J, Vavrova V, Kuzelova M, Ticha J, Prusa R. Serum cystatin C level for better assessment of glomerular filtration rate in cystic fibrosis patients treated by amikacin. *J Clin Pharm Ther* 2008; **33**: 409-417.

Hanaoka K, Devuyst O, Schwiebert EM, Wilson PD, Guggino WB. A role for CFTR in human autosomal dominant polycystic kidney disease. *Am J Physiol* 1996; **270**: C389-399.

Hayashi M, Numaguchi M, Watabe H, Enomoto H, Yaoi Y. Cisplatin-induced nephrotoxicity and the protective effect of fosfomycin on it as demonstrated by using a crossover study of urinary metabolite levels. *Acta Obstet Gynecol Scand* 1997; **76**: 590-595.

Heeckeren A, Walenga R, Konstan MW, Bonfield T, Davis PB, Ferkol T. Excessive inflammatory response of cystic fibrosis mice to bronchopulmonary infection with *Pseudomonas aeruginosa*. *J Clin Invest* 1997; **100**: 2810-2815.

Heinzl B, Eber E, Oberwaldner B, Haas G, Zach MS. Effects of inhaled gentamicin prophylaxis on acquisition of *Pseudomonas aeruginosa* in children with cystic fibrosis: a pilot study. *Pediatr Pulmonol* 2002; **33**: 32-37.

Hendlin D, Stapley EO, Jackson M, Wallick H, Miller AK, Wolf FJ, Miller TW, Chaiet L, Kahan FM, Foltz EL, Woodruff HB, Mata JM, Hernandez S, Mochales S. Phosphonomycin, a new antibiotic produced by strains of streptomyces. *Science* 1969; **166**: 122-123.

Herber RFM. Beta-2 microglobulin and other urinary proteins as an index of cadmium-nephrotoxicity. *Pure Appl Chem* 1984; **56**: 957-965.

Hinshaw HC, Feldman WH. Streptomycin in the treatment of tuberculosis: a preliminary report. *Mayo Clin Proc* 1945; **20**: 313-318.

Hmiel SP, Beck AM, de la Morena MT, Sweet S. Progressive chronic kidney disease after pediatric lung transplantation. *Am J Transplant* 2005; **5**: 1739-1747.

HO SA, Lee TWR, Denton M, Conway S. Succesful antibiotic eradication of *Pseudomonas aeruginosa* infection does not promote drug resistance in subsequest re-growths of this bacterium in children. *J Cyst Fibros* 2004; **3**: S34.

Hodson ME, Penketh AR, Batten JC. Aerosol carbenicillin and gentamicin treatment of *Pseudomonas aeruginosa* infection in patients with cystic fibrosis. *Lancet* 1981; **2**: 1137-1139.

Hodson ME, Gallagher CG, Govan JR. A randomised clinical trial of nebulised tobramycin or colistin in cystic fibrosis. *Eur Respir J* 2002; **20**: 658-664.

Hoffler D, Koeppe P, Paeske B. Pharmacokinetics of azithromycin in normal and impaired renal function. *Infection* 1995; **23**: 356-361.

Hoffmann IM, Rubin BK, Iskandar SS, Schechter MS, Nagaraj SK, Bitzan MM. Acute renal failure in cystic fibrosis: association with inhaled tobramycin therapy. *Pediatr Pulmonol* 2002; **34**: 375-377.

Hofmann W, Guder WG. A diagnostic programme for quantitative analysis of proteinuria. *J Clin Chem Clin Biochem* 1989; **27**: 589-600.

Holdt-Lehmann B, Lehmann A, Korten G, Nagel H, Nizze H, Schuff-Werner P. Diagnostic value of urinary alanine aminopeptidase and N-acetyl-beta-D-glucosaminidase in comparison to alpha 1-microglobulin as a marker in evaluating tubular dysfunction in glomerulonephritis patients. *Clin Chim Acta* 2000; **297**: 93-102.

Hong CY, Chia KS. Markers of diabetic nephropathy. *J Diabetes Complications* 1998; **12**: 43-60.

Hoppe B, Hesse A, Bromme S, Rietschel E, Michalk D. Urinary excretion substances in patients with cystic fibrosis: risk of urolithiasis? *Pediatr Nephrol* 1998; **12**: 275-279.

Hoppe B, von Unruh GE, Blank G, Rietschel E, Sidhu H, Laube N, Hesse A. Absorptive hyperoxaluria leads to an increased risk for urolithiasis or nephrocalcinosis in cystic fibrosis. *Am J Kidney Dis* 2005; **46**: 440-445.

Hoyle BD, Costerton JW. Bacterial resistance to antibiotics: the role of biofilms. *Prog Drug Res* 1991; **37**: 91-105.

Huber S, Braun G, Burger-Kentischer A, Reinhart B, Luckow B, Horster M. CFTR mRNA and its truncated splice variant (TRN-CFTR) are differentially expressed during collecting duct ontogeny. *FEBS Lett* 1998; **423**: 362-366.

Hugli R, Artho G, Wiesmann UN, Peheim E, Schaad UB, Bianchetti MG. Assessment of the nephrotoxicity of amikacin in patients with cystic fibrosis. *Schweiz Med Wochenschr* 1992; **122**: 930-935.

Hultberg B, Ravnskov U. The excretion of N-acetyl-beta-glucosaminidase in glomerulonephritis. *Clin Nephrol* 1981; **15**: 33-38.

Husted RF, Volk KA, Sigmund RD, Stokes JB. Anion secretion by the inner medullary collecting duct. Evidence for involvement of the cystic fibrosis transmembrane conductance regulator. *J Clin Invest* 1995; **95**: 644-650.

Ikeda A, Konta T, Takasaki S, Hao Z, Suzuki K, Sato H, Shibata Y, Takeishi Y, Kato T, Kawata S, Kubota I. In a non-diabetic Japanese population, the combination of macroalbuminuria and increased urine beta 2-microglobulin predicts a decline of renal function: the Takahata study. *Nephrol Dial Transplant* 2009; **24**: 841-847.

Inouye S, Niizato T, Komiya I, Yuda Y, Yamada Y. Mode of protective action of fosfomycin against dibekacin-induced nephrotoxicity in the dehydrated rats. *J Pharmacobiodyn* 1982; **5**: 941-950.

Ishani A, Erturk S, Hertz MI, Matas AJ, Savik K, Rosenberg ME. Predictors of renal function following lung or heart-lung transplantation. *Kidney Int* 2002; **61**: 2228-2234.

Ismailov, II, Awayda MS, Jovov B, Berdiev BK, Fuller CM, Dedman JR, Kaetzel M, Benos DJ. Regulation of epithelial sodium channels by the cystic fibrosis transmembrane conductance regulator. *J Biol Chem* 1996; **271**: 4725-4732.

Jardin A. A general practitioner multicenter study: fosfomycin trometamol single dose versus pipemidic acid multiple dose. *Infection* 1990; **18 Suppl 2**: S89-93.

Jelliffe RW. Estimation of creatinine clearance when urine can not be collected. *Lancet* 1971; **1**: 975-976.

Jelliffe RW. Letter: Creatinine clearance: bedside estimate. *Ann Intern Med* 1973; **79**: 604-605.

Jensen T, Pedersen SS, Hoiby N, Koch C, Flensborg EW. Use of antibiotics in cystic fibrosis. The Danish approach. *Antibiot Chemother* 1989; **42**: 237-246.

Johansen HK, Nir M, Hoiby N, Koch C, Schwartz M. Severity of cystic fibrosis in patients homozygous and heterozygous for delta F508 mutation. *Lancet* 1991; **337**: 631-634.

Johansen HK, Norregaard L, Gotzsche PC, Pressler T, Koch C, Hoiby N. Antibody response to Pseudomonas aeruginosa in cystic fibrosis patients: a marker of therapeutic success?--A 30-year cohort study of survival in Danish CF patients after onset of chronic P. aeruginosa lung infection. *Pediatr Pulmonol* 2004; **37**: 427-432.

John R, Herzenberg AM. Renal toxicity of therapeutic drugs. *J Clin Pathol* 2009; **62**: 505-515.

Jones AM, Govan JR, Doherty CJ, Dodd ME, Isalska BJ, Stanbridge TN, Webb AK. Spread of a multiresistant strain of *Pseudomonas aeruginosa* in an adult cystic fibrosis clinic. *Lancet* 2001; **358**: 557-558.

Jung K, Scholz D. An optimized assay of alanine aminopeptidase activity in urine. *Clin Chem* 1980; **26**: 1251-1254.

Junge W, Wilke B, Halabi A, Jarausch J, Klein G. Reference intervals for total protein in collected and random urine using the benzothonium chloride method. *Clin Chem* 2006; **52**: A157.

Kahan FM, Kahan JS, Cassidy PJ, Kropp H. The mechanism of action of fosfomycin (phosphonomycin). *Ann N Y Acad Sci* 1974; **235**: 364-386.

Kalansooriya A, Jennings P, Haddad F, Holbrook I, Whiting PH. Urinary enzyme measurements as early indicators of renal insult in type 2 diabetes. *Br J Biomed Sci* 2007; **64**: 153-156.

Kallel H, Hamida CB, Ksibi H, Bahloul M, Hergafi L, Chaari A, Chelly H, Bouaziz M. Suspected acute interstitial nephritis induced by colistin. *J Nephrol* 2005; **18**: 323-326.

Kaloyanides G. Aminoglycoside nephrotoxicity. In: Diseases of the Kidney, Fifth Edition. Schrier R, Gottaschalk C, eds. Boston: Little Brown & Co., 1992: 1131-1163.

Kanga J, Kuhn R, Craigmyle L, Haverstock D, Church D. Cystic fibrosis clinical score: a new scoring system to evaluate acute pulmonary exacerbation. *Clin Ther* 1999; **21**: 1343-1356.

Kasiakou SK, Michalopoulos A, Soteriades ES, Samonis G, Sermaides GJ, Falagas ME. Combination therapy with intravenous colistin for management of infections due to

multidrug-resistant Gram-negative bacteria in patients without cystic fibrosis. *Antimicrob Agents Chemother* 2005; **49**: 3136-3146.

Katz SM, Krueger LJ, Falkner B. Microscopic nephrocalcinosis in cystic fibrosis. *N Engl J Med* 1988; **319**: 263-266.

Kennedy SE, Henry RL, Rosenberg AR. Antibiotic-related renal failure and cystic fibrosis. *J Paediatr Child Health* 2005; **41**: 382-383.

Kerem B, Rommens JM, Buchanan JA, Markiewicz D, Cox TK, Chakravarti A, Buchwald M, Tsui LC. Identification of the cystic fibrosis gene: genetic analysis. *Science* 1989; **245**: 1073-1080.

Khan TZ, Wagener JS, Bost T, Martinez J, Accurso FJ, Riches DW. Early pulmonary inflammation in infants with cystic fibrosis. *Am J Respir Crit Care Med* 1995; **151**: 1075-1082.

Kinai E, Hanabusa H. Renal tubular toxicity associated with tenofovir assessed using urine-beta 2 microglobulin, percentage of tubular reabsorption of phosphate and alkaline phosphatase levels. *AIDS* 2005; **19**: 2031-2033.

Kizer NL, Vandorpe D, Lewis B, Bunting B, Russell J, Stanton BA. Vasopressin and cAMP stimulate electrogenic chloride secretion in an IMCD cell line. *Am J Physiol* 1995; **268**: F854-861.

Koch C, Hoiby N. Pathogenesis of cystic fibrosis. *Lancet* 1993; **341**: 1065-1069.

Koch C, Hjelt K, Pedersen SS, Jensen ET, Jensen T, Lanng S, Valerius NH, Pedersen M, Hoiby N. Retrospective clinical study of hypersensitivity reactions to aztreonam and six other beta-lactam antibiotics in cystic fibrosis patients receiving multiple treatment courses. *Rev Infect Dis* 1991; **13 Suppl 7**: S608-611.

Koeppen BM, Stanton BA. Sodium chloride transport: distal nephron. In: The Kidney: physiology and pathophysiology, second Edition. Seldin DW, Giebisch G, eds. New York: Raven Press, 1992: 2003-2039.

Konstan MW, Berger M. Current understanding of the inflammatory process in cystic fibrosis: onset and etiology. *Pediatr Pulmonol* 1997; **24**: 137-142; discussion 159-161.

Konstan MW, Butler SM, Schidlow DV, Morgan WJ, Julius JR, Johnson CA. Patterns of medical practice in cystic fibrosis: part II. Use of therapies. Investigators and Coordinators of the Epidemiologic Study of Cystic Fibrosis. *Pediatr Pulmonol* 1999; **28**: 248-254.

Kovesi TA, Swartz R, MacDonald N. Transient renal failure due to simultaneous ibuprofen and aminoglycoside therapy in children with cystic fibrosis. *N Engl J Med* 1998; **338**: 65-66.

Kumon H, Ono N, Iida M, Nickel JC. Combination effect of fosfomycin and ofloxacin against *Pseudomonas aeruginosa* growing in a biofilm. *Antimicrob Agents Chemother* 1995; **39**: 1038-1044.

Kumon H, Tomochika K, Matunaga T, Ogawa M, Ohmori H. A sandwich cup method for the penetration assay of antimicrobial agents through Pseudomonas exopolysaccharides. *Microbiol Immunol* 1994; **38**: 615-619.

Kunin CM, Chesney RW, Craig WA, England AC, DeAngelis C. Enzymuria as a marker of renal injury and disease: studies of N-acetyl-beta-glucosaminidase in the general population and in patients with renal disease. *Pediatrics* 1978; **62**: 751-760.

Kuwertz-Broking E, Koch HG, Schulze Everding A, Bulla M, Dworinczak B, Helmchen U, Harms E. Colchicine for secondary nephropathic amyloidosis in cystic fibrosis. *Lancet* 1995; **345**: 1178-1179.

Lafrance JP, Miller DR. Selective and non-selective non-steroidal anti-inflammatory drugs and the risk of acute kidney injury. *Pharmacoepidemiol Drug Saf* 2009.

Landman D, Georgescu C, Martin DA, Quale J. Polymyxins revisited. *Clin Microbiol Rev* 2008; **21**: 449-465.

Lang B, Hauk P, Meske S, Keller E, Peter HH. Kidney function in therapy with non-steroidal antiphlogistic drugs. A double-blind cross-over study with diclofenac, indomethacin and piroxicam. *Z Rheumatol* 1991; **50**: 366-370.

Lang BJ, Aaron SD, Ferris W, Hebert PC, MacDonald NE. Multiple combination bactericidal antibiotic testing for patients with cystic fibrosis infected with multiresistant strains of *Pseudomonas aeruginosa*. *Am J Respir Crit Care Med* 2000; **162**: 2241-2245.

Lebecque P, Leal T, Zylberberg K, Reychler G, Bossuyt X, Godding V. Towards zero prevalence of chronic *Pseudomonas aeruginosa* infection in children with cystic fibrosis. *J Cyst Fibros* 2006; **5**: 237-244.

Ledson MJ, Gallagher MJ, Hart CA, Walshaw MJ. Differential effect of antibiotic therapy on colony counts and inflammatory markers in CF patients colonised with *B. cepacia* or *P. aeruginosa*. *Paediatr pulmonol* 1999; **suppl 19**: 274.

Ledson MJ, Gallagher MJ, Cowperthwaite C, Convery RP, Walshaw MJ. Four years' experience of intravenous colomycin in an adult cystic fibrosis unit. *Eur Respir J* 1998; **12**: 592-594.

Lee TW, Brownlee KG, Denton M, Littlewood JM, Conway SP. Reduction in prevalence of chronic *Pseudomonas aeruginosa* infection at a regional pediatric cystic fibrosis center. *Pediatr Pulmonol* 2004; **37**: 104-110.

Letz B, Korbmacher C. cAMP stimulates CFTR-like Cl- channels and inhibits amiloride-sensitive Na+ channels in mouse CCD cells. *Am J Physiol* 1997; **272**: C657-666.

Levey AS, Perrone RD, Madias NE. Serum creatinine and renal function. *Annu Rev Med* 1988; **39**: 465-490.

Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med* 1999; **130**: 461-470.

Levy J, Smith AL, Kenny MA, Ramsey B, Schoenknecht FD. Bioactivity of gentamicin in purulent sputum from patients with cystic fibrosis or bronchiectasis: comparison with activity in serum. *J Infect Dis* 1983; **148**: 1069-1076.

Levy J, Smith AL, Koup JR, Williams-Warren J, Ramsey B. Disposition of tobramycin in patients with cystic fibrosis: a prospective controlled study. *J Pediatr* 1984; **105**: 117-124.

Li Z, Kosorok MR, Farrell PM, Laxova A, West SE, Green CG, Collins J, Rock MJ, Splaingard ML. Longitudinal development of mucoid *Pseudomonas aeruginosa* infection and lung disease progression in children with cystic fibrosis. *JAMA* 2005; **293**: 581-588.

Ling BN, Kokko KE, Eaton DC. Prostaglandin E2 activates clusters of apical Cl-channels in principal cells via a cyclic adenosine monophosphate-dependent pathway. *J Clin Invest* 1994; **93**: 829-837.

LiPuma JJ. Microbiological and immunologic considerations with aerosolized drug delivery. *Chest* 2001; **120**: 118S-123S.

Littlewood JM, Miller MG, Ghoneim AT, Ramsden CH. Nebulised colomycin for early pseudomonas colonisation in cystic fibrosis. *Lancet* 1985; **1**: 865.

Littlewood JM, Koch C, Lambert PA, Hoiby N, Elborn JS, Conway SP, Dinwiddie R, Duncan-Skingle F. A ten year review of colomycin. *Respir Med* 2000; **94**: 632-640.

Ljutic D, Rumboldt Z. Possible interaction between azithromycin and cyclosporin: a case report. *Nephron* 1995; **70**: 130.

Lloyd SE, Pearce SH, Fisher SE, Steinmeyer K, Schwappach B, Scheinman SJ, Harding B, Bolino A, Devoto M, Goodyer P, Rigden SP, Wrong O, Jentsch TJ, Craig IW, Thakker RV. A common molecular basis for three inherited kidney stone diseases. *Nature* 1996; **379**: 445-449.

Lomaestro BM. Fluoroquinolone-induced renal failure. *Drug Saf* 2000; **22**: 479-485.

Lopez-Cerero L, de Cueto M, Diaz-Guerrero MA, Morillo C, Pascual A. Evaluation of the Etest method for fosfomycin susceptibility of ESBL-producing *Klebsiella pneumoniae*. *J Antimicrob Chemother* 2007; **59**: 810-812.

Lott RS, Hayton WL. Estimation of creatinine clearance from serum creatinine concentration. *Drug Intell Clin Pharm* 1978; **12**: 40-50.

Luke RG. Natriuresis and chloruresis during hydrogenia in the rat. *Am J Physiol* 1973; **224**: 13-20.

Lyczak JB, Cannon CL, Pier GB. Lung infections associated with cystic fibrosis. *Clin Microbiol Rev* 2002; **15**: 194-222.

MacGowan AP, Bailey RA, Egner W, Picken DM, Reeves DS. An open study of the efficacy and safety of single dose fosfomycin trometamol in treatment of hospitalised patients with urinary tract infection (pilot study). *Infection* 1990; **18 Suppl 2**: S107-108.

Magryta CJ, Hennigar R, Weatherly M. Pathological case of the month. Diabetic nephropathy in cystic fibrosis. *Arch Pediatr Adolesc Med* 1999; **153**: 1307-1308.

Mansoor GA, Panner BJ, Ornt DB. Azithromycin-induced acute interstitial nephritis. *Ann Intern Med* 1993; **119**: 636-637.

Marchewka Z, Dlugosz A. Enzymes in urine as markers of nephrotoxicity of cytostatic agents and aminoglycoside antibiotics. *Int Urol Nephrol* 1998; **30**: 339-348.

Marvao P, De Jesus Ferreira MC, Bailly C, Paulais M, Bens M, Guinamard R, Moreau R, Vandewalle A, Teulon J. Cl- absorption across the thick ascending limb is not altered in cystic fibrosis mice. A role for a pseudo-CFTR Cl- channel. *J Clin Invest* 1998; **102**: 1986-1993.

Master V, Roberts GW, Coulthard KP, Baghurst PA, Martin A, Roberts ME, Onishko CR, Martin AJ, Linke RJ, Holmes M, Jarvinen A, Kennedy D, Colebatch KA, Hansman D, Parsons DW. Efficacy of once-daily tobramycin monotherapy for acute pulmonary exacerbations of cystic fibrosis: a preliminary study. *Pediatr Pulmonol* 2001; **31**: 367-376.

Matthews I, Kirkpatrick C, Holford N. Quantitative justification for target concentration intervention--parameter variability and predictive performance using population pharmacokinetic models for aminoglycosides. *Br J Clin Pharmacol* 2004; **58**: 8-19.

Matthews LA, Doershuk CF, Stern RC, Resnick MI. Urolithiasis and cystic fibrosis. *J Urol* 1996; **155**: 1563-1564.

Mayama T, Yokota M, Shimatani I, Ohyagi H. Analysis of oral fosfomycin calcium (Fosmicin) side-effects after marketing. *Int J Clin Pharmacol Ther Toxicol* 1993; **31**: 77-82.

McGlennen RC, Burke BA, Dehner LP. Systemic amyloidosis complicating cystic fibrosis. A retrospective pathologic study. *Arch Pathol Lab Med* 1986; **110**: 879-884.

McKone EF, Emerson SS, Edwards KL, Aitken ML. Effect of genotype on phenotype and mortality in cystic fibrosis: a retrospective cohort study. *Lancet* 2003; **361**: 1671-1676.

McNicholas CM, Guggino WB, Schwiebert EM, Hebert SC, Giebisch G, Egan ME. Sensitivity of a renal K+ channel (ROMK2) to the inhibitory sulfonylurea compound glibenclamide is enhanced by coexpression with the ATP-binding cassette transporter cystic fibrosis transmembrane regulator. *Proc Natl Acad Sci U S A* 1996; **93**: 8083-8088.

Mearns MB. Aerosol therapy in cystic fibrosis. *Arch Dis Child* 1970; **45**: 605-607.

Melzi ML, Costantini D, Giani M, Appiani AC, Giunta AM. Severe nephropathy in three adolescents with cystic fibrosis. *Arch Dis Child* 1991; **66**: 1444-1447.

Mendelman PM, Smith AL, Levy J, Weber A, Ramsey B, Davis RL. Aminoglycoside penetration, inactivation, and efficacy in cystic fibrosis sputum. *Am Rev Respir Dis* 1985; **132**: 761-765.

Miller M, Ward L, Thomas BJ, Cooksley WG, Shepherd RW. Altered body composition and muscle protein degradation in nutritionally growth-retarded children with cystic fibrosis. *Am J Clin Nutr* 1982; **36**: 492-499.

Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CP, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas D, Pedersen OF, Pellegrino R, Viegi G, Wanger J. Standardisation of spirometry. *Eur Respir J* 2005; **26**: 319-338.

Mingeot-Leclercq MP, Tulkens PM. Aminoglycosides: nephrotoxicity. *Antimicrob Agents Chemother* 1999; **43**: 1003-1012.

Mirahmadi MK, Byrne C, Barton C, Penera N, Gordon S, Vaziri ND. Prediction of creatinine clearance from serum creatinine in spinal cord injury patients. *Paraplegia* 1983; **21**: 23-29.

Mirakhur A, Gallagher MJ, Ledson MJ, Hart CA, Walshaw MJ. Fosfomycin therapy for multiresistant *Pseudomonas aeruginosa* in cystic fibrosis. *J Cyst Fibros* 2003; **2**: 19-24.

Mondorf AW. Urinary enzymatic markers of renal damage. In: The aminoglycosides. Whelton A, Neu HC, eds. New York: Marcel Dekker, 1982: 283-303.

Mondorf AW, Heynold FT, Scherberich JE, Hess H, Schoeppe W. Assessment of the nephrotoxic potential of ceftazidime and a ceftazidime/tobramycin combination in volunteers. *Infection* 1983; **11 Suppl 1**: S57-62.

Moore JE, Crowe M, Shaw A, McCaughan J, Redmond AO, Elborn JS. Antibiotic resistance in *Burkholderia cepacia* at two regional cystic fibrosis centres in Northern Ireland: is there a need for synergy testing? *J Antimicrob Chemother* 2001; **48**: 319-321.

Morales MM, Capella MA, Lopes AG. Structure and function of the cystic fibrosis transmembrane conductance regulator. *Braz J Med Biol Res* 1999; **32**: 1021-1028.

Morales MM, Falkenstein D, Lopes AG. The cystic fibrosis transmembrane regulator (CFTR) in the kidney. *An Acad Bras Cienc* 2000; **72**: 399-406.

Morales MM, Nascimento DS, Capella MA, Lopes AG, Guggino WB. Arginine vasopressin regulates CFTR and ClC-2 mRNA expression in rat kidney cortex and medulla. *Pflugers Arch* 2001; **443**: 202-211.

Morales MM, Carroll TP, Morita T, Schwiebert EM, Devuyst O, Wilson PD, Lopes AG, Stanton BA, Dietz HC, Cutting GR, Guggino WB. Both the wild type and a functional isoform of CFTR are expressed in kidney. *Am J Physiol* 1996; **270**: F1038-1048.

Morin JP, Bendirdjian JP, Fillastre JP, Vaillant R. Interference of fosfomycin with the integrity of the lysosomal membrane of rat kidney cells. *Minerva Med* 1978; **69**: 4075-4078.

Morin JP, Olier B, Viotte G, Fillastre JP. Can fosfomycin reduce the nephrotoxicity of aminoglycosides?. *Pathol Biol (Paris)* 1984; **32**: 338-342.

Morosini MI, Garcia-Castillo M, Loza E, Perez-Vazquez M, Baquero F, Canton R. Breakpoints for predicting *Pseudomonas aeruginosa* susceptibility to inhaled tobramycin in cystic fibrosis patients: use of high-range Etest strips. *J Clin Microbiol* 2005; **43**: 4480-4485.

Morris AP. The regulation of epithelial cell cAMP- and calcium-dependent chloride channels. *Adv Pharmacol* 1999; **46**: 209-251.

Morris AP, Frizzell RA. Vesicle targeting and ion secretion in epithelial cells: implications for cystic fibrosis. *Annu Rev Physiol* 1994; **56**: 371-397.

Moss RB. Administration of aerosolized antibiotics in cystic fibrosis patients. *Chest* 2001; **120**: 107S-113S.

Moss RB. Long-term benefits of inhaled tobramycin in adolescent patients with cystic fibrosis. *Chest* 2002; **121**: 55-63.

Moyer BD, McCoy DE, Lee B, Kizer N, Stanton BA. Adenosine inhibits arginine vasopressin-stimulated chloride secretion in a mouse IMCD cell line (mIMCD-K2). *Am J Physiol* 1995; **269**: F884-891.

Mueller PW, Smith SJ, Steinberg KK, Thun MJ. Chronic renal tubular effects in relation to urine cadmium levels. *Nephron* 1989; **52**: 45-54.

Mukhopadhyay S, Singh M, Cater JI, Ogston S, Franklin M, Olver RE. Nebulised antipseudomonal antibiotic therapy in cystic fibrosis: a meta-analysis of benefits and risks. *Thorax* 1996; **51**: 364-368.

Mussap M, Plebani M. Biochemistry and clinical role of human cystatin C. *Crit Rev Clin Lab Sci* 2004; **41**: 467-550.

Nadesalingam K, Conway SP, Denton M. Risk factors for acquisition of methicillin-resistant *Staphylococcus aureus* (MRSA) by patients with cystic fibrosis. *J Cyst Fibros* 2005; **4**: 49-52.

National Institue for Health and Clinical Excellence. Chronic Kidney Disease: Early identification and managment of chronic kidney disease in adults in primary and secondary care. NICE clinical guideline 73, September 2008: available from www.nice.org.uk/CG073, accessed 28 Jan 2010.

National Kidney Foundation. K/DOQI Clinical practice guidelines for chronic Kidney disease: evaluation, classification and stratification. . *Am J Kidney Dis* 2002; **39 (Suppl 1)**: S1-S266.

Nicoll SR, Sainsbury R, Bailey RR, King A, Frampton C, Elliot JR, Turner JG. Assessment of creatinine clearance in healthy subjects over 65 years of age. *Nephron* 1991; **59**: 621-625.

Nix DE, Thomas JK, Symonds WT, Spivey JM, Wilton JH, Gagliardi NC, Schentag JJ. Assessment of the enzymuria resulting from gentamicin alone and combinations of

gentamicin with various beta-lactam antibiotics. *Ann Pharmacother* 1997; **31**: 696-703.

Ohtani I, Ohtsuki K, Aikawa T, Sato Y, Anzai T, Ouchi J. Mechanism of protective effect of fosfomycin against aminoglycoside ototoxicity. *Auris Nasus Larynx* 1984; **11**: 119-124.

Ohtani I, Ohtsuki K, Aikawa T, Sato Y, Anzai T, Ouchi J, Saito T. Protective effect of fosfomycin against aminoglycoside ototoxicity. *ORL J Otorhinolaryngol Relat Spec* 1985; **47**: 42-48.

Orme ML. Non-steroidal anti-inflammatory drugs and the kidney. *Br Med J (Clin Res Ed)* 1986; **292**: 1621-1622.

Orr A, McVean RJ, Webb AK, Dodd ME. Questionnaire survey of urinary incontinence in women with cystic fibrosis. *BMJ* 2001; **322**: 1521.

Patzer L. Nephrotoxicity as a cause of acute kidney injury in children. *Pediatr Nephrol* 2008; **23**: 2159-2173.

Pedersen SS, Jensen T, Osterhammel D, Osterhammel P. Cumulative and acute toxicity of repeated high-dose tobramycin treatment in cystic fibrosis. *Antimicrob Agents Chemother* 1987; **31**: 594-599.

Perea EJ, Daza RM, Mendaza MP. Genetic localization of the resistance to fosfomycin. *Chemotherapy* 1977; **23 Suppl 1**: 127-132.

Perez-Brayfield MR, Caplan D, Gatti JM, Smith EA, Kirsch AJ. Metabolic risk factors for stone formation in patients with cystic fibrosis. *J Urol* 2002; **167**: 480-484.

Perkins BA, Nelson RG, Ostrander BE, Blouch KL, Krolewski AS, Myers BD, Warram JH. Detection of renal function decline in patients with diabetes and normal or elevated GFR by serial measurements of serum cystatin C concentration: results of a 4-year follow-up study. *J Am Soc Nephrol* 2005; **16**: 1404-1412.

Pitt TL, Sparrow M, Warner M, Stefanidou M. Survey of resistance of *Pseudomonas aeruginosa* from UK patients with cystic fibrosis to six commonly prescribed antimicrobial agents. *Thorax* 2003; **58**: 794-796.

Preston SL, Briceland LL, Lomaestro BM, Lesar TS, Bailie GR, Drusano GL. Dosing adjustment of 10 antimicrobials for patients with renal impairment. *Ann Pharmacother* 1995; **29**: 1202-1207.

Price DJ, Graham DI. Effects of large doses of colistin sulphomethate sodium on renal function. *Br Med J* 1970; **4**: 525-527.

Price RG. Urinary enzymes, nephrotoxicity and renal disease. *Toxicology* 1982; **23**: 99-134.

Prime D, Tune B. The nephrotoxicity of antimicrobial drugs. In: Pediatrics Update. Moss A, ed. New York: Elsevier/North Holland, 1981: 265-285.

Pusey CD, Saltissi D, Bloodworth L, Rainford DJ, Christie JL. Drug associated acute interstitial nephritis: clinical and pathological features and the response to high dose steroid therapy. *Q J Med* 1983; **52**: 194-211.

Ramsey BW, Pepe MS, Quan JM, Otto KL, Montgomery AB, Williams-Warren J, Vasiljev KM, Borowitz D, Bowman CM, Marshall BC, Marshall S, Smith AL. Intermittent administration of inhaled tobramycin in patients with cystic fibrosis. Cystic Fibrosis Inhaled Tobramycin Study Group. *N Engl J Med* 1999; **340**: 23-30.

Randall RV, Goldstein NP, Gross JB, Rosevear JW, McGuckin WF. Effect of Adrenocortical Steroids on the Hypercalciuria of Wilson's Disease (Hepatolenticular Degeneration). *Metabolism* 1965; **14**: 12-16.

Ratjen F, Doring G. Cystic fibrosis. *Lancet* 2003; **361**: 681-689.

Ratjen F, Doring G, Nikolaizik WH. Effect of inhaled tobramycin on early *Pseudomonas aeruginosa* colonisation in patients with cystic fibrosis. *Lancet* 2001; **358**: 983-984.

Ratjen F, Rietschel E, Kasel D, Schwiertz R, Starke K, Beier H, van Koningsbruggen S, Grasemann H. Pharmacokinetics of inhaled colistin in patients with cystic fibrosis. *J Antimicrob Chemother* 2006; **57**: 306-311.

Reed MD, Vermeulen MW, Stern RC, Cheng PW, Powell SH, Boat TF. Are measurements of urine enzymes useful during aminoglycoside therapy? *Pediatr Res* 1981; **15**: 1234-1239.

Reeves DS. Fosfomycin trometamol. *J Antimicrob Chemother* 1994; **34**: 853-858.

Regelmann WE, Elliott GR, Warwick WJ, Clawson CC. Reduction of sputum *Pseudomonas aeruginosa* density by antibiotics improves lung function in cystic fibrosis more than do bronchodilators and chest physiotherapy alone. *Am Rev Respir Dis* 1990; **141**: 914-921.

Rey E, Treluyer JM, Pons G. Drug disposition in cystic fibrosis. *Clin Pharmacokinet* 1998; **35**: 313-329.

Rhodes PJ, Rhodes RS, McClelland GH, Culbertson VL, Jahnigen DW, Bloedow DC. Evaluation of eight methods for estimating creatinine clearance in men. *Clin Pharm* 1987; **6**: 399-406.

Ring E, Eber E, Erwa W, Zach MS. Urinary N-acetyl-beta-D-glucosaminidase activity in patients with cystic fibrosis on long-term gentamicin inhalation. *Arch Dis Child* 1998; **78**: 540-543.

Riordan JR. The cystic fibrosis transmembrane conductance regulator. *Annu Rev Physiol* 1993; **55**: 609-630.

Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak Z, Zielenski J, Lok S, Plavsic N, Chou JL, et al. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* 1989; **245**: 1066-1073.

Rocha AS, Kudo LH. Factors governing sodium and chloride transport across the inner medullary collecting duct. *Kidney Int* 1990; **38**: 654-667.

Rojanasthien N, Kumsorn B, Atikachai B, Leotrakul S, Thongprasert S. Protective effects of fosfomycin on cisplatin-induced nephrotoxicity in patients with lung cancer. *Int J Clin Pharmacol Ther* 2001; **39**: 121-125.

Rommens JM, Iannuzzi MC, Kerem B, Drumm ML, Melmer G, Dean M, Rozmahel R, Cole JL, Kennedy D, Hidaka N, et al. Identification of the cystic fibrosis gene: chromosome walking and jumping. *Science* 1989; **245**: 1059-1065.

Rosenfeld M, Emerson J, Williams-Warren J, Pepe M, Smith A, Montgomery AB, Ramsey B. Defining a pulmonary exacerbation in cystic fibrosis. *J Pediatr* 2001a; **139**: 359-365.

Rosenfeld M, Gibson RL, McNamara S, Emerson J, Burns JL, Castile R, Hiatt P, McCoy K, Wilson CB, Inglis A, Smith A, Martin TR, Ramsey BW. Early pulmonary infection, inflammation, and clinical outcomes in infants with cystic fibrosis. *Pediatr Pulmonol* 2001b; **32**: 356-366.

Rowe SM, Miller S, Sorscher EJ. Cystic fibrosis. *N Engl J Med* 2005; **352**: 1992-2001.

Rubera I, Tauc M, Bidet M, Poujeol C, Cuiller B, Watrin A, Touret N, Poujeol P. Chloride currents in primary cultures of rabbit proximal and distal convoluted tubules. *Am J Physiol* 1998; **275**: F651-663.

Rubera I, Tauc M, Verheecke-Mauze C, Bidet M, Poujeol C, Touret N, Cuiller B, Poujeol P. Regulation of cAMP-dependent chloride channels in DC1 immortalized rabbit distal tubule cells in culture. *Am J Physiol* 1999; **276**: F104-121.

Rule AD, Larson TS, Bergstralh EJ, Slezak JM, Jacobsen SJ, Cosio FG. Using serum creatinine to estimate glomerular filtration rate: accuracy in good health and in chronic kidney disease. *Ann Intern Med* 2004; **141**: 929-937.

Ryan G, Mukhopadhyay S, Singh M. Nebulised anti-pseudomonal antibiotics for cystic fibrosis. *Cochrane Database Syst Rev* 2003: CD001021.

Rybak MJ, Frankowski JJ, Edwards DJ, Albrecht LM. Alanine aminopeptidase and beta 2-microglobulin excretion in patients receiving vancomycin and gentamicin. *Antimicrob Agents Chemother* 1987; **31**: 1461-1464.

Saiman L, Mehar F, Niu WW, Neu HC, Shaw KJ, Miller G, Prince A. Antibiotic susceptibility of multiply resistant *Pseudomonas aeruginosa* isolated from patients with cystic fibrosis, including candidates for transplantation. *Clin Infect Dis* 1996; **23**: 532-537.

Salazar DE, Corcoran GB. Predicting creatinine clearance and renal drug clearance in obese patients from estimated fat-free body mass. *Am J Med* 1988; **84**: 1053-1060.

Samaniego-Picota MD, Whelton A. Aminoglycoside-Induced Nephrotoxicity in Cystic Fibrosis: A Case Presentation and Review of the Literature. *Am J Ther* 1996; **3**: 248-257.

Sampson MJ, Drury PL. Accurate estimation of glomerular filtration rate in diabetic nephropathy from age, body weight, and serum creatinine. *Diabetes Care* 1992; **15**: 609-612.

Sawicki GS, Rasouliyan L, Ren CL. The impact of MRSA on lung function in patients with cystic fibrosis. *Am J Respir Crit Care Med* 2009; **179**: 734-735; author reply 735.

Schaad UB, Wedgwood-Krucko J, Suter S, Kraemer R. Efficacy of inhaled amikacin as adjunct to intravenous combination therapy (ceftazidime and amikacin) in cystic fibrosis. *J Pediatr* 1987; **111**: 599-605.

Scheinberg P, Shore E. A pilot study of the safety and efficacy of tobramycin solution for inhalation in patients with severe bronchiectasis. *Chest* 2005; **127**: 1420-1426.

Schiavina G, Arrigo G, Vergnaghi D, Cavaliere G, Scarduelli B, Luzzatti G, D'Amico G. Nephrotoxicity of contrast media. Comparison of iopamidol and diatrizoate by measurement of urinary enzymes after urography. *Radiol Med* 1984; **70**: 176-179.

Schindler R, Radke C, Paul K, Frei U. Renal problems after lung transplantation of cystic fibrosis patients. *Nephrol Dial Transplant* 2001; **16**: 1324-1328.

Schulin T. In vitro activity of the aerosolized agents colistin and tobramycin and five intravenous agents against *Pseudomonas aeruginosa* isolated from cystic fibrosis patients in southwestern Germany. *J Antimicrob Chemother* 2002; **49**: 403-406.

Schwarzenberg SJ, Thomas W, Olsen TW, Grover T, Walk D, Milla C, Moran A. Microvascular complications in cystic fibrosis-related diabetes. *Diabetes Care* 2007; **30**: 1056-1061.

Schwiebert EM, Egan ME, Hwang TH, Fulmer SB, Allen SS, Cutting GR, Guggino WB. CFTR regulates outwardly rectifying chloride channels through an autocrine mechanism involving ATP. *Cell* 1995; **81**: 1063-1073.

Scott CS, Retsch-Bogart GZ, Henry MM. Renal failure and vestibular toxicity in an adolescent with cystic fibrosis receiving gentamicin and standard-dose ibuprofen. *Pediatr Pulmonol* 2001; **31**: 314-316.

Scott F, Pitt T. Identification and characterization of transmissible *Pseudomonas aeruginosa* strains in cystic fibrosis centres in Great Britain. *Pediatr Pulmonol [Suppl]* 2003; **25**: 307.

Scott FW, Pitt TL. Identification and characterization of transmissible *Pseudomonas aeruginosa* strains in cystic fibrosis patients in England and Wales. *J Med Microbiol* 2004; **53**: 609-615.

Selvadurai HC, Allen J, Sachinwalla T, Macauley J, Blimkie CJ, Van Asperen PP. Muscle function and resting energy expenditure in female athletes with cystic fibrosis. *Am J Respir Crit Care Med* 2003; **168**: 1476-1480.

Semsarian C. Efficacy of inhaled tobramycin in cystic fibrosis. *J Paediatr Child Health* 1990; **26**: 110-111.

Sethi K, Diamond LH. Aminoglycoside nephrotoxicity and its predictability. *Nephron* 1981; **27**: 265-270.

Shemesh O, Golbetz H, Kriss JP, Myers BD. Limitations of creatinine as a filtration marker in glomerulopathic patients. *Kidney Int* 1985; **28**: 830-838.

Sidhu H, Hoppe B, Hesse A, Tenbrock K, Bromme S, Rietschel E, Peck AB. Absence of *Oxalobacter formigenes* in cystic fibrosis patients: a risk factor for hyperoxaluria. *Lancet* 1998; **352**: 1026-1029.

Sierra F, Suarez M, Rey M, Vela MF. Systematic review: Proton pump inhibitor-associated acute interstitial nephritis. *Aliment Pharmacol Ther* 2007; **26**: 545-553.

Simpson JE, Gawenis LR, Walker NM, Boyle KT, Clarke LL. Chloride conductance of CFTR facilitates basal Cl-/HCO3- exchange in the villous epithelium of intact murine duodenum. *Am J Physiol Gastrointest Liver Physiol* 2005; **288**: G1241-1251.

Smith AL, Fiel SB, Mayer-Hamblett N, Ramsey B, Burns JL. Susceptibility testing of Pseudomonas aeruginosa isolates and clinical response to parenteral antibiotic administration: lack of association in cystic fibrosis. *Chest* 2003; **123**: 1495-1502.

Smith AL, Ramsey BW, Hedges DL, Hack B, Williams-Warren J, Weber A, Gore EJ, Redding GJ. Safety of aerosol tobramycin administration for 3 months to patients with cystic fibrosis. *Pediatr Pulmonol* 1989; **7**: 265-271.

Smith AL, Redding G, Doershuk C, Goldmann D, Gore E, Hilman B, Marks M, Moss R, Ramsey B, Rubio T, et al. Sputum changes associated with therapy for endobronchial exacerbation in cystic fibrosis. *J Pediatr* 1988; **112**: 547-554.

Smith C, Coulthard K, Turnidge J, Smith G, Martin AJ, Henning P. Renal function monitoring for IV tobramycin and colistin in patients with cystic fibrosis. *Abstract 7th Conference Proceedings ASA* 2006.

Smith JJ, Travis SM, Greenberg EP, Welsh MJ. Cystic fibrosis airway epithelia fail to kill bacteria because of abnormal airway surface fluid. *Cell* 1996; **85**: 229-236.

Smyth A, Lewis S, Bertenshaw C, Choonara I, McGaw J, Watson A. Case-control study of acute renal failure in patients with cystic fibrosis in the UK. *Thorax* 2008; **63**: 532-535.

Smyth A, Tan KH, Hyman-Taylor P, Mulheran M, Lewis S, Stableforth D, Prof Knox A. Once versus three-times daily regimens of tobramycin treatment for pulmonary exacerbations of cystic fibrosis--the TOPIC study: a randomised controlled trial. *Lancet* 2005; **365**: 573-578.

Smyth AR, Tan KH. Once-daily versus multiple-daily dosing with intravenous aminoglycosides for cystic fibrosis. *Cochrane Database Syst Rev* 2006; **3**: CD002009.

Soulsby N, Bell S, Greville H, Doecke C. Intravenous aminoglycoside usage and monitoring of patients with cystic fibrosis in Australia. What's new? *Intern Med J* 2009; **39**: 527-531.

Spencker FB, Staber L, Lietz T, Schille R, Rodloff AC. Development of resistance in *Pseudomonas aeruginosa* obtained from patients with cystic fibrosis at different times. *Clin Microbiol Infect* 2003; **9**: 370-379.

Steinkamp G, Tummler B, Malottke R, von der Hardt H. Treatment of *Pseudomonas aeruginosa* colonisation in cystic fibrosis. *Arch Dis Child* 1989; **64**: 1022-1028.

Steinkamp G, Lutge M, Wurster U, Schulz-Baldes JG, Grone HJ, Ehrich JH. Renal function in cystic fibrosis: proteinuria and enzymuria before and after tobramycin therapy. *Eur J Pediatr* 1986; **145**: 526-531.

Stenvinkel P, Hjelte L, Alvan G, Hedman A, Hultman E, Strandvik B. Decreased renal clearance of sodium in cystic fibrosis. *Acta Paediatr Scand* 1991; **80**: 194-198.

Stephens D, Garey N, Isles A, Levison H, Gold R. Efficacy of inhaled tobramycin in the treatment of pulmonary exacerbations in children with cystic fibrosis. *Pediatr Infect Dis* 1983; **2**: 209-211.

Stephens SE, Rigden SP. Cystic fibrosis and renal disease. *Paediatr Respir Rev* 2002; **3**: 135-138.

Stettler N, Kawchak DA, Boyle LL, Propert KJ, Scanlin TF, Stallings VA, Zemel BS. Prospective evaluation of growth, nutritional status, and body composition in children with cystic fibrosis. *Am J Clin Nutr* 2000; **72**: 407-413.

Stevens LA, Coresh J, Schmid CH, Feldman HI, Froissart M, Kusek J, Rossert J, Van Lente F, Bruce RD, 3rd, Zhang YL, Greene T, Levey AS. Estimating GFR using serum cystatin C alone and in combination with serum creatinine: a pooled analysis of 3,418 individuals with CKD. *Am J Kidney Dis* 2008; **51**: 395-406.

Stirati G, Antonelli M, Fofi C, Fierimonte S, Pecci G. IgA nephropathy in cystic fibrosis. *J Nephrol* 1999; **12**: 30-31.

Stosic Z, Sedlak V, Felle D, Tepavcevic P, Jovanovic D, Ubavic M. The importance of beta-2 microglobulin determination in the urine in the detection of tubular proteinuria. *Med Pregl* 1989; **42**: 318-321.

Strandvik B, Hjelte L. Nephrolithiasis in cystic fibrosis. *Acta Paediatr* 1993; **82**: 306-307.

Strandvik B, Berg U, Kallner A, Kusoffsky E. Effect on renal function of essential fatty acid supplementation in cystic fibrosis. *J Pediatr* 1989; **115**: 242-250.

Stutts MJ, Canessa CM, Olsen JC, Hamrick M, Cohn JA, Rossier BC, Boucher RC. CFTR as a cAMP-dependent regulator of sodium channels. *Science* 1995; **269**: 847-850.

Sullivan LP, Wallace DP, Grantham JJ. Chloride and fluid secretion in polycystic kidney disease. *J Am Soc Nephrol* 1998; **9**: 903-916.

Sullivan MM, Denning CR. Diabetic microangiopathy in patients with cystic fibrosis. *Pediatrics* 1989; **84**: 642-647.

Taccetti G, Campana S, Festini F, Mascherini M, Doring G. Early eradication therapy against *Pseudomonas aeruginosa* in cystic fibrosis patients. *Eur Respir J* 2005; **26**: 458-461.

Takahashi K, Kanno H. Synergistic activities of combinations of beta-lactams, fosfomycin, and tobramycin against *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 1984; **26**: 789-791.

Tan KH, Mulheran M, Knox AJ, Smyth AR. Aminoglycoside prescribing and surveillance in cystic fibrosis. *Am J Respir Crit Care Med* 2003; **167**: 819-823.

Tan KH, Hyman-Tylor P, Mulheran M, Knox A, Smyth A. Lack of concordance in the use and monitoring of intravenous aminoglycosides in UK cystic fibrosis centers. *Pediatr Pulmonol* 2002a; **33**: 165.

Tan KH, Hyman-Taylor P, Mulheran M, Knox A, Smyth A. Once-daily tobramycin monotherapy in cystic fibrosis. *Pediatr Pulmonol* 2002b; **33**: 406; author reply 407.

Tauc M, Bidet M, Poujeol P. Chloride currents activated by calcitonin and cAMP in primary cultures of rabbit distal convoluted tubule. *J Membr Biol* 1996; **150**: 255-273.

Terribile M, Capuano M, Cangiano G, Carnovale V, Ferrara P, Petrarulo M, Marangella M. Factors increasing the risk for stone formation in adult patients with cystic fibrosis. *Nephrol Dial Transplant* 2006; **21**: 1870-1875.

Tessier F, Quentin C. In vitro activity of fosfomycin combined with ceftazidime, imipenem, amikacin, and ciprofloxacin against *Pseudomonas aeruginosa*. *Eur J Clin Microbiol Infect Dis* 1997; **16**: 159-162.

Thakur V, Reisin E, Solomonow M, Baratta R, Aguilar E, Best R, D'Ambrosia R, Solanky T. Accuracy of formula-derived creatinine clearance in paraplegic subjects. *Clin Nephrol* 1997; **47**: 237-242.

Todd-Turla KM, Rusvai E, Naray-Fejes-Toth A, Fejes-Toth G. CFTR expression in cortical collecting duct cells. *Am J Physiol* 1996; **270**: F237-244.

Touw DJ, Knox AJ, Smyth A. Population pharmacokinetics of tobramycin administered thrice daily and once daily in children and adults with cystic fibrosis. *J Cyst Fibros* 2007; **6**: 327-333.

Touw DJ, Vinks AA, Heijerman HG, Hermans J, Bakker W. Suggestions for the optimization of the initial tobramycin dose in adolescent and adult patients with cystic fibrosis. *Ther Drug Monit* 1994; **16**: 125-131.

Touw DJ, Brimicombe RW, Hodson ME, Heijerman HG, Bakker W. Inhalation of antibiotics in cystic fibrosis. *Eur Respir J* 1995; **8**: 1594-1604.

Touw DJ, Jacobs FA, Brimicombe RW, Heijerman HG, Bakker W, Briemer DD. Pharmacokinetics of aerosolized tobramycin in adult patients with cystic fibrosis. *Antimicrob Agents Chemother* 1997; **41**: 184-187.

Town DJ, Vinks AA, Jacobs F, Heijerman HG, Bakker W. Creatinine clearance as predictor of tobramycin elimination in adult patients with cystic fibrosis. *Ther Drug Monit* 1996; **18**: 562-569.

Trof RJ, Di Maggio F, Leemreis J, Groeneveld AB. Biomarkers of acute renal injury and renal failure. *Shock* 2006; **26**: 245-253.

Trollfors B, Alestig K, Krantz I, Norrby R. Quantitative nephrotoxicity of gentamicin in nontoxic doses. *J Infect Dis* 1980; **141**: 306-309.

Tschakert H, Matern-Pinzek R, Schaffeldt J. Increase of kidney enzymes in urine after administration of contrast media: comparison of the nephrotoxicity of ionic and nonionic substances. *Aktuelle Radiol* 1995; **5**: 152-156.

Turner MA, Goldwater D, David TJ. Oxalate and calcium excretion in cystic fibrosis. *Arch Dis Child* 2000; **83**: 244-247.

Tyner M. Investigation of elevated urine beta-2-microglobulin in a cohort of cadmium workers. *J Occup Environ Med* 1999; **41**: 745-746.

Ullmann U, Lindemann B. In vitro investigations on the action fosfomycin alone and in combination with other antibiotics on Pseudomonas aeruginosa and Serratia marcescens. *Arzneimittelforschung* 1980; **30**: 1247-1249.

Umeki S, Watanabe M, Yagi S, Soejima R. Supplemental fosfomycin and/or steroids that reduce cisplatin-induced nephrotoxicity. *Am J Med Sci* 1988; **295**: 6-10.

Valerius NH, Koch C, Hoiby N. Prevention of chronic *Pseudomonas aeruginosa* colonisation in cystic fibrosis by early treatment. *Lancet* 1991; **338**: 725-726.

van den Berg JM, Morton AM, Kok SW, Pijl H, Conway SP, Heijerman HG. Microvascular complications in patients with cystic fibrosis-related diabetes (CFRD). *J Cyst Fibros* 2008; **7**: 515-519.

Vazquez C, Municio M, Corera M, Gaztelurrutia L, Sojo A, Vitoria JC. Early treatment of *Pseudomonas aeruginosa* colonization in cystic fibrosis. *Acta Paediatr* 1993; **82**: 308-309.

Venaille TJ, Ryan G, Robinson BW. Epithelial cell damage is induced by neutrophil-derived, not pseudomonas-derived, proteases in cystic fibrosis sputum. *Respir Med* 1998; **92**: 233-240.

Viau C, Bernard A, Ouled A, Lauwerys R. Determination of rat beta 2-microglobulin in urine and in serum. II. Application of its urinary measurement to selected nephrotoxicity models. *J Appl Toxicol* 1986; **6**: 191-195.

Viergever PP, Swaak AJ. Urine- and serum beta 2-microglobulin in patients with rheumatoid arthritis: a study of 101 patients without signs of kidney disease. *Clin Rheumatol* 1989; **8**: 368-374.

Vinton NE, Padman R, Davis M, Harcke HT. Effects of Pseudomonas colonization on body composition and resting energy expenditure in children with cystic fibrosis. *JPEN J Parenter Enteral Nutr* 1999; **23**: 233-236.

von der Heiden R, Balestra AP, Bianchetti MG, Casaulta Aebischer C, Mullis PE, Lippuner K, Jaeger P. Which factors account for renal stone formation in cystic fibrosis? *Clin Nephrol* 2003; **59**: 160-163.

Wang W. Regulation of the ROMK channel: interaction of the ROMK with associate proteins. *Am J Physiol* 1999; **277**: F826-831.

Waz WR, Stapleton FB, Borowitz D. Clinical quiz. Renal amyloidosis. *Pediatr Nephrol* 1995; **9**: 245-246.

Weber A, Morlin G, Cohen M, Williams-Warren J, Ramsey B, Smith A. Effect of nebulizer type and antibiotic concentration on device performance. *Pediatr Pulmonol* 1997; **23**: 249-260.

Westall GP, Binder J, Kotsimbos T, Topliss D, Thomson N, Dowling J, Wilson JW. Nodular glomerulosclerosis in cystic fibrosis mimics diabetic nephropathy. *Nephron Clin Pract* 2004; **96**: c70-75.

Whelton A. Renal effects of over-the-counter analgesics. *J Clin Pharmacol* 1995; **35**: 454-463.

Whitehead A, Conway SP, Etherington C, Caldwell NA, Setchfield N, Bogle S. Once-daily tobramycin in the treatment of adult patients with cystic fibrosis. *Eur Respir J* 2002; **19**: 303-309.

Wibell L. The serum level and urinary excretion of beta2-microglobulin in health and renal disease. *Pathol Biol (Paris)* 1978; **26**: 295-301.

Wilson PD. Epithelial cell polarity and disease. *Am J Physiol* 1997; **272**: F434-442.

Winter MC, Sheppard DN, Carson MR, Welsh MJ. Effect of ATP concentration on CFTR Cl- channels: a kinetic analysis of channel regulation. *Biophys J* 1994; **66**: 1398-1403.

Wood PJ, Ioannides-Demos LL, Li SC, Williams TJ, Hickey B, Spicer WJ, Hooper RE, McLean AJ. Minimisation of aminoglycoside toxicity in patients with cystic fibrosis. *Thorax* 1996; **51**: 369-373.

Woodland C, Blowey D, Ito S, Spino M, Koren G. Hypothetical framework for enhanced renal tubular secretion of drugs in cystic fibrosis. *Med Hypotheses* 1998; **51**: 489-491.

Woodruff HB, Mata JM, Hernandez S, Mochales S, Rodriguez A, Stapley EO, Wallick H, Miller AK, Hendlin D. Fosfomycin: Laboratory studies. *Chemotherapy* 1977; **23 Suppl 1**: 1-22.

Yuen CT, Price RG, Chattagoon L, Richardson AC, Praill PF. Colorimetric assays for N-acetyl-beta-D-glucosaminidase and beta-D-galactosidase in human urine using newly-developed omega-nitrostyryl substrates. *Clin Chim Acta* 1982; **124**: 195-204.

Zahran A, El-Husseini A, Shoker A. Can cystatin C replace creatinine to estimate glomerular filtration rate? A literature review. *Am J Nephrol* 2007; **27**: 197-205.

Zielenski J, Rozmahel R, Bozon D, Kerem B, Grzelczak Z, Riordan JR, Rommens J, Tsui LC. Genomic DNA sequence of the cystic fibrosis transmembrane conductance regulator (CFTR) gene. *Genomics* 1991; **10**: 214-228.